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The ecology and control of earthworms on golf courses

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This thesis is submitted in fulfilment of the requirement for the degree of Doctor of Philosophy.

For my father, who really should do one of his own...

Abstract

Earthworm casts on golf courses affect the playability of the turf and can potentially damage mowing equipment. Traditionally this problem has been limited using chemical controls. It is estimated that 0.6% of the total UK land surface is occupied by golf courses, therefore, the land management strategies which green keepers adopt with respect to the application of chemicals has a major environmental impact. The aim of this thesis was to investigate the ecology and potential control of earthworm casting in golf turf in environmentally sustainable ways.

A quadrat survey of earthworm casts was conducted over two years at five golf courses in Bedfordshire and Buckinghamshire, UK. Using generalized linear models and forward multiple stepwise regression, an internally validated predictive model of earthworm casting activity was constructed. Annual activity on surfaces was predicted using five physicochemical parameters of which C:N and total inorganic nitrogen were the most important. Environmental parameters were also used to predict monthly earthworm activity, with evapotranspiration and rainfall representing the most significant variation.

Mustard extraction surveys were used to investigate species diversity and community structure of earthworms. Four dominant species were identified (*Aporrectodea rosea*, *Lumbricus rubellus*, *Aporrectodea longa* and *Lumbricus terrestris*). It is likely that *A. longa* and *L. terrestris*, the two must abundant anecic forms, cause the greatest problems to green keepers as these are the largest of the four earthworm species.

The microbial community of soil represents the earthworm's primary food source. An analysis of the microbial community size (using chloroform-extraction) and community structure (using phospholipid fatty acid [PLFA] analysis) showed that different surfaces found on golf courses supported significantly distinct and consistent microbial communities. Differences in population size and structure were evident at different depths through all golf course soil profiles investigated. Individual surface types were comparable, irrespective of geographical location. Therefore different surfaces and depths through the soil profile on golf courses represent different earthworm habitats.

An investigation of the effects of different construction techniques and materials used in the golf industry on the rate of earthworm cast formation was made. This showed no effect of construction on the vertical distribution of earthworms, but the rate of casting increased on the sand dominated surfaces. It is proposed that this is due to the lower calorific value that this soil represents to the earthworms. This knowledge was applied in an earthworm cast mitigation experiment, reducing casting rates by stimulating the size of the microbial community with glucose solution. Control through physical exclusion of earthworms to the surface using a buried mesh was also trialled and significantly reduced earthworm casts, however no causal mechanism could be elucidated.

This study has advanced the understanding of earthworm ecology on golf courses, deriving mechanistic understandings of this system as a whole. This will lead to a more environmentally sustainable approach to the control of earthworms on golf courses.

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Definition of terms

- **Aeration** – The mechanical disruption of the soil without destroying the turfs, for example using hollow tining or slitting in order to reduce compaction and increase the movement of air and water through the soil.
- **Anthropogenic soil horizon** – A man-made soil profile as defined by the World Reference Base for soils.
- **Aggregates** – Soil particles held tightly together, either by surface attraction forces (hydrogen bonding and Van der Waals forces) and gums, mucilage and hyphae generated by the biologically active component of the soil, forming a basic soil structural unit.
- **Bulk density** – The mass of dry soil material per unit volume.
- **Fairway** – The area of a golf course that links the tee to the green over which each hole is (ideally) played.
- **Green** – The area of a golf course where the hole is located, with closely cut grass.
    - **Standard green** – A green built by ramping the surrounding soil up into a levelled mound. Sometimes with a perched water table.
    - **USGA specification green** – A green built to a high specification using closely defined root zone material with a perched water table.
- **K selected life-style strategy** – A species that has a long life cycle, with a low growth rate and low fecundity.
- **Pedogenesis** – The formation of soil profiles dependent on five soil forming factors; climate, parent material, topography, organisms, and time.
- **r selected life-style strategy** – A species that has a short life cycle, with a fast growth rate and a high fecundity and able to utilise ready assimilable resources only.
- **Soil profile** – The vertical arrangement of layers of soil down to the bedrock.
- **Soil macrofauna** – The animal life-forms found in soil that are above the microscopic scale, such as earthworms (>2 mm).

- **Soil microfauna** – Life-forms found in the soil that are smaller than the microscopic scales, such as bacteria (1-2 μm), fungi (2-10 μm) and nematodes (0.3 - 8000 μm).
- **Suspended water table** – A water table separated from the main water table beneath it by a zone that is not saturated.
- **Tee** – The starting place for each hole on a golf course.
    - **Standard Tee** – The standard construction method for tees on golf course: an area is closely mown and top dressed with sand to produce a regulated starting point for each hole.
    - **USGA specification tee** – This tee type is constructed using USGA specification root zone at the surface to increase drainage and promote better turf growth.
- **Top dressing** – The application of sand and soil mixes to the surface of greens and tees used to alleviate surface compaction, smooth uneven surfaces and aid the decomposition of thatch.
- **Thatch** – The build up of decomposing root and plant material between the growing tips and the soil surface, caused by an in balance of nutrient cycling.
- **USGA** – United States Golf Association
- **USGA specification root zone** – This is a root zone mixture of between 80 – 90% sand and the remainder as peat or soil, produced to strict specifications with regards particle size analysis.

Chapter 1: Introduction

1. 1. Overview

In the UK, sport is of great social and economic importance. Communities are unified by the support of clubs and teams; individuals are taught the importance of team-work in achieving a games goal; or pent up aggression is acceptably discharged through the rules of a specific game. UK sport is supported by central government and the National Lottery, through organisations such as Sport England (Sport England Staff 2006). Since its inception in 1997 this organisation has invested £2,200,000,000 of lottery derived funds and £550,000,000 from the Exchequer in 2006. The current round of investment is set to distribute £100,000,000 into 30 key sports[1] between 2003 – 2006 (Russell 2006). The distribution of spending in sport is not even. Sports with greater popularity and easier access by individuals, such as football and athletics, command a greater proportion of spending from governmental organisations than private individuals, and as such government funding is deliberately focused on large participant sports where communities would be enhanced by access to facilities and equipment (Figure 1.1). Most golf clubs are private companies, with significant revenues and relatively poor community access, which means that they are generally illegible for government funding. Golf as a sport is of major importance to an aging population allowing retired individuals to maintain an active lifestyle.

[1] One of the 30 key sports specified by Sport England is golf.

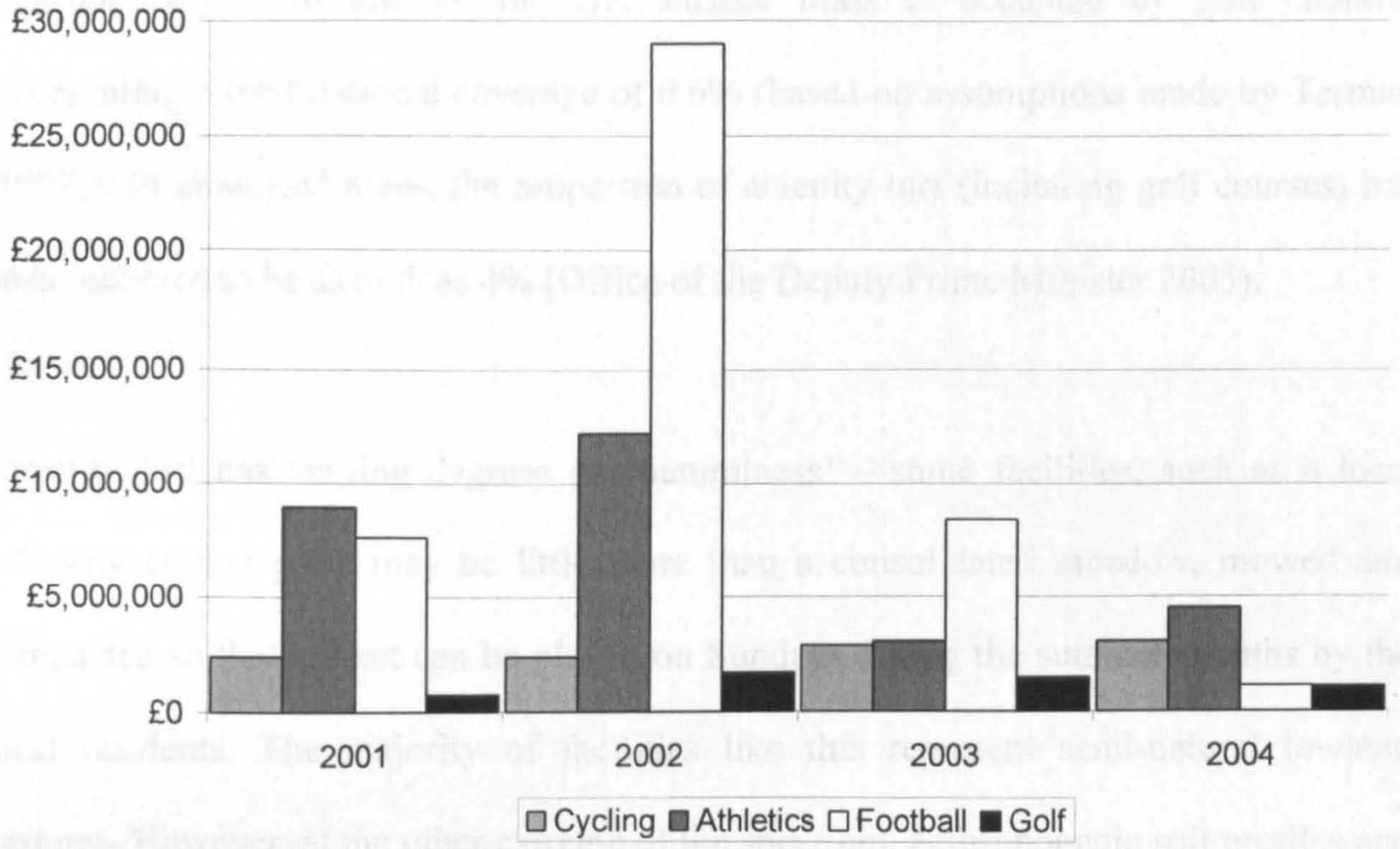


Figure 1.1: Histogram showing Sport England investment in four different sports between 2001 and 2004. Data derived from Sport England website (Sport England Staff 2006)

A large proportion of the UK's gross domestic produce can be associated in some way with sport, through the multi-million pound industries from agrochemicals and turf care products, to the shoe, equipment and clothing industries. Individuals, clubs and facilities are run as businesses on the back of sporting endeavours. All Premier League football clubs are traded as high-value public limited companies; the last time that Manchester United Football Club plc was valued its net worth was estimated at £741,000,000. Similarly, golf courses and facilities are generally either private or publically limited companies, with clubs having retail values of upwards of £7,500,000 (Agriseek Staff 2006). Investment in golf courses is frequently on an individual basis, with organisations such as Polaris World building integrated golf courses and residential facilities for private purchase (Polaris World Staff 2006). It is estimated that

approximately 1500 km^2 of the UK surface mass is occupied by golf courses, representing a total national coverage of 0.6% (based on assumptions made by Terman (1997)). In urbanised areas, the proportion of amenity turf (including golf courses) has been recorded to be as high as 4% (Office of the Deputy Prime Minister 2005).

Amenity turf has varying degrees on 'naturalness' – some facilities, such as a local authority cricket pitch may be little more than a consolidated meadow, mowed and compacted so that cricket can be played on Sundays during the summer months by the local residents. The majority of facilities like this represent semi-natural lowland pastures. However, at the other extreme of the spectrum, anthropogenic soil profiles and radically altered grass species compositions are found at some facilities e.g. high specification golf greens on courses that host international competitions. In the case of the Lords cricket ground, these man-made soil profile and grass species constructions represent the whole facility (ECB staff 2006).

There is little known about the soil biology of sports facilities, and the ecology of earthworms in sports turf is an area of research that has received very little attention. The majority of studies of earthworms have been carried out in arable, pasture land (Edwards and Lofty 1977; Curry 1998) or forests (Lee 1985), but the soil environmental conditions in these systems are considerably different from the anthropogenic soil materials used to create the engineered soil profiles that are found below sports surfaces. Golf courses are intensively managed grassland systems, with regular irrigation and mowing, coupled with the application of a wide variety of fertilizers and biocides to promote desirable turf growth. There is also considerable difference between the grass

species present and species diversity that are found on golf courses and natural grasslands. *Poa annua* (annual meadow grass), frequently found in pastureland is considered as a weed on golf courses where mown ground cover is maintained as exclusively fine-leaved grass species (Balogh and Walker 1992). These differences mean that direct comparison and application of the knowledge of earthworm activity and diversity from other grassland ecosystems may not be appropriate. Further investigation into the problems, and their solutions, in a golf course context is therefore required.

Earthworms, the soil's natural eco-system engineer (Lavelle 2001), can affect the playability at sports facilities by producing casts on the surface of turfgrass. Earthworm casts can lead to a decline in terms of overall aesthetics and trueness of ball roll. Smeared earthworm casts on the surface of these recreational amenities are not considered attractive by most participants in, or spectators of, sports. The earthworm casts can also cause damage to mowing and other maintenance equipment, presenting significant management problems for greenkeepers. A poor management strategy of earthworm populations can decrease the perceived playability of a golf course and thus impact upon its profitability. Earthworm species that cast soil on the surface of golf courses have been causing problems to green keepers since the game was devised, sometime in the 13th Century (McGrath 2006). Several labour intensive solutions to remove the result of this problem (such as switching and brushing) have been used since then. More recently, chemical controls have been used. The most effective and widely used chemical, chlordane, was banned in 1992 following changes to the pesticide regulations (Baldwin and Bennett 1990; Perris 1996a). Several fungicides used for

fungal control, can also be used at higher application rates as a vermicide but only have a short lived effect (Woolhouse and Wright 1984; Baker *et al.* 1998). All other methods to control earthworms aim to manipulate the soil conditions to generate an ostensibly hostile soil environment for earthworms (Cook *et al.* 1997; Baker *et al.* 2000; Williamson and Hong 2005). These have only had limited success because hostile conditions for earthworms are also hostile to, and generally retard, grass growth. There is therefore a pressing need for effective but environmentally benign methods to control earthworm casting on golf courses, balancing legislative and business requirements. A suitable control mechanism that can be scaled up for whole golf course use could also be financially valuable to the golf industry.

1. 2. Review of literature

Earthworms are invertebrates, found within the class Oligochaeta in the phylum Annelida[2]. This phylum comprises approximately 15,000 species, and terrestrial oligochaete worms account for around 3,700 of these species (Brusca and Brusca 1990). Fossil evidence shows that earthworms began to colonise terrestrial environments about 500 million years ago, during the Cambrian explosion, and they are now the most predominant species in the soil macro-fauna (Lavelle 2001). In the UK, 25 species of earthworms are found and their distribution is related to geography, local soil conditions and microclimate. Most communities have between eight and ten species making up the population structure (Brusca and Brusca 1990; Curry 1998).

As well as being the most predominant macrofauna, earthworms are one of the most important in UK soils. This is because of their role in pedogenesis and soil profile

[2] The phylum Annelida consists of all segmented worms.

development (Brady and Weil 1999). An earthworm will ingest between 2 - 30 times its body weight in soil per day. This action has major impacts on the drainage properties and fertility of the soil. The burrows of earthworms in some soils can account for up to 9 L m^{-3} of the pore space in volume of soil (Lavelle *et al.* 1987), and earthworms can also account for the cycling of up to 100 kg N ha^{-1} y^{-1} in woodland ecosystems as earthworm biomass (Curry 1987).

1. 2. 1. The drilosphere

The actions of earthworms in soils, and their significance has been considered for over 120 years. The first published work, by Darwin (1883) "*The formation of vegetable mould, through the action of worms*" was a seminal overview of the ecology and habitats where earthworms are found in temperate environments. The understanding of the role of earthworms in the terrestrial environment has been advanced considerably since that time.

The concept of the drilosphere was introduced by Lavelle *et al.* (1987), a term that encompasses the area of influence of the earthworm (in the same way that the rhizosphere describes the area affected by the roots of plants). In this way the whole soil system can be considered with the earthworm, including localised interactions with soil bacteria and fungi. The drilosphere was defined as the soil within a 2 mm vicinity of a earthworm, accounting for roughly 3% of the soil volume. It can contain between 5-25% of soil microflora by volume (Lavelle *et al.* 1987).

In a typical base rich, fertile soil the fresh weight mass of earthworms can be greater than 100 g m^{-2} at the surface. While earthworms are very important in the development

of the soil profile, they actually contribute very little to primary litter decomposition. This is because they lack the gut enzymes to digest cellulose, hemicellulose and other complex sugars (Lee 1985). They do however promote litter mixing from which bacterial decomposition benefits (Curry 1987). There is also an increase in the assimilation of phosphorus, potassium, calcium, and magnesium by crop plants in earthworm populated soils (Curry 1987; Postma-Blaauw *et al.* 2006).

Earthworms are very adaptive creatures and can live in a range of soil environments. Diversification is apparent within and between species. Consequently three distinct ecological groups can be distinguished (Lee 1985; Lavelle *et al.* 1987):

1. **Epigeic earthworms:** These species of earthworms feed on and live in the leaf litter. They are unable to burrow into the soil and are most commonly found in woodland environments. These species normally have a high fecundity and short life expectancy (r type life-style strategy).
2. **Anecic earthworms:** This ecological classification of earthworm is capable of burrowing and normally have burrows that are open at the surface. They feed on leaf litter that they find at the surface and mix it within the soil horizons. This type of earthworm is responsible for the formation of surface casts that cause major problems within the sports turf industry. They have a slow growth rate and a low fecundity (K type life-style strategy).
3. **Endogeic earthworms:** These earthworms also form burrows but they are not open to the surface. They live in the upper soil horizons (normally the top 15 to 20 cm) – species representing this niche follow both r or K life-style strategies.

Of the 25 species of earthworm found in the UK, only three are frequently reported as adopting anecic life-styles. It has been demonstrated that these three species can all cause casting problems on sports turfs; *Allolophora cholorotica* (Savigny) will feed on deep roots; *Aporrectodea longa* (Ude) will feed on leaf litter; *Lumbricus terrestris* (L.) feeds on a combination of deep roots and leaf litter (Lee 1985; Brusca and Brusca 1990).

These different ecological groupings and the different habitats of earthworms mean that not all species present a problem with respect to surface casting in sports turf. Only anecic species cast at the surface, endogeic species are actually beneficial to the turf environment, increasing drainage and promoting decomposition of thatch. Thatch is defined as the layer of living and dead organic matter that occurs between the green biomass and the soil surface, caused by an imbalance between inputs and outputs of plant biomass, frequently instigated by the overuse of inorganic nitrogenous fertilisers (Potter *et al.* 1990).

1. 2. 2. Earthworm physiology

Earthworms are one of the most ancient terrestrial animal groups and within the 3,700 species the basic body plan varies very little. It essentially consists of two concentric tubes, one creating the body wall and the other the gut. They are separated by a fluid filled cavity, called the coelom (Figure 1.2). This whole structure is divided into a series of structural segments, of which there may be hundreds of in a single worm. These segments and the structures that are found as part of them, such as the position of the male pores or the length of the tubercula pubertais, are taxonomically pertinent (Figure 1.3).

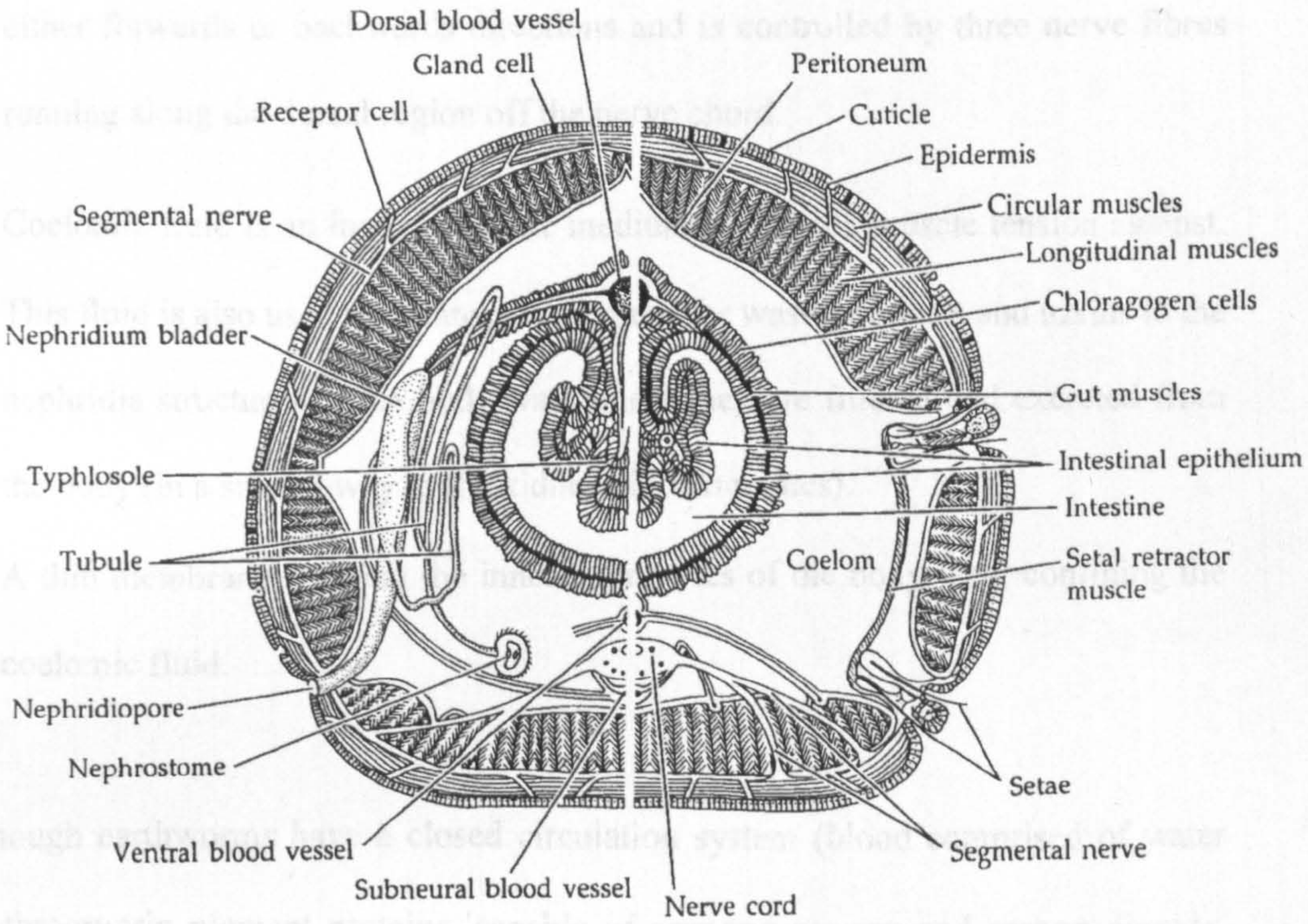


Figure 1.2: Stylised cross section of oligochaete body wall. Left hand side of diagram shows a single nephridium (composite of two segments); the right hand side shows the setae and associated muscle structure (adapted from Brusca and Brusca 1990).

Several studies have analysed the cross sectional structural components of a range of earthworms (Lee 1985). Generically they consist of:

- A tough but thin, laminated cuticle made from collagen (therefore unstretchable). The arrangement of the long chain molecules in this layer results in a fibrous 'skeleton'.
- An epidermis consisting of supporting column cells that attach to the cuticle.
- A layer of circular and longitudinal muscle fibres. The muscles are mutually antagonistic and allow movement by producing pressure against the coelomic fluid. Locomotion is coordinated between each segment in sequence, by

alternate contractions of the circular and longitudinal muscle. This is possible in either forwards or backwards directions and is controlled by three nerve fibres running along the dorsal region off the nerve chord.

- Coelomic fluid is an incompressible medium to provide muscle tension against. This fluid is also used as a transport medium for waste products and toxins to the nephridia structures in the body wall where they are filtered and excreted from the body (in a similar way to the kidneys in vertebrates).
- A thin membrane defining the inner boundaries of the body wall, confining the coelomic fluid.

Even though earthworms have a closed circulation system (blood comprised of water and erythrocruorin pigment proteins, capable of carrying oxygen and carbon dioxide, around the body in dedicated vessels) earthworms have no dedicated body parts for gaseous exchange (Lee 1985; Brusca and Brusca 1990). Blood vessels run underneath the cuticle and mucus is excreted to the exterior to aid diffusion. This mucus also helps with lubrication and locomotion. The respiratory gases must diffuse into the circulatory system across the whole body surface. For this reason the surface area to volume ratio is a key factor in defining both body shape and size. Most worm species have a surface area to volume ratio of between two and four (Brusca and Brusca 1990). The respiration rate for *L. terrestris* is approximately 38.7 to 45.2 mm^3 O_2 h^{-1} g body $weight^{-1}$ at 10°C and considerably higher at increased temperatures. Normal respiratory behaviour is seen in atmospheres of up to 50% CO_2. Earthworms are also capable of anaerobic respiration and can survive for several hours in anoxic environments (Edwards 1996).

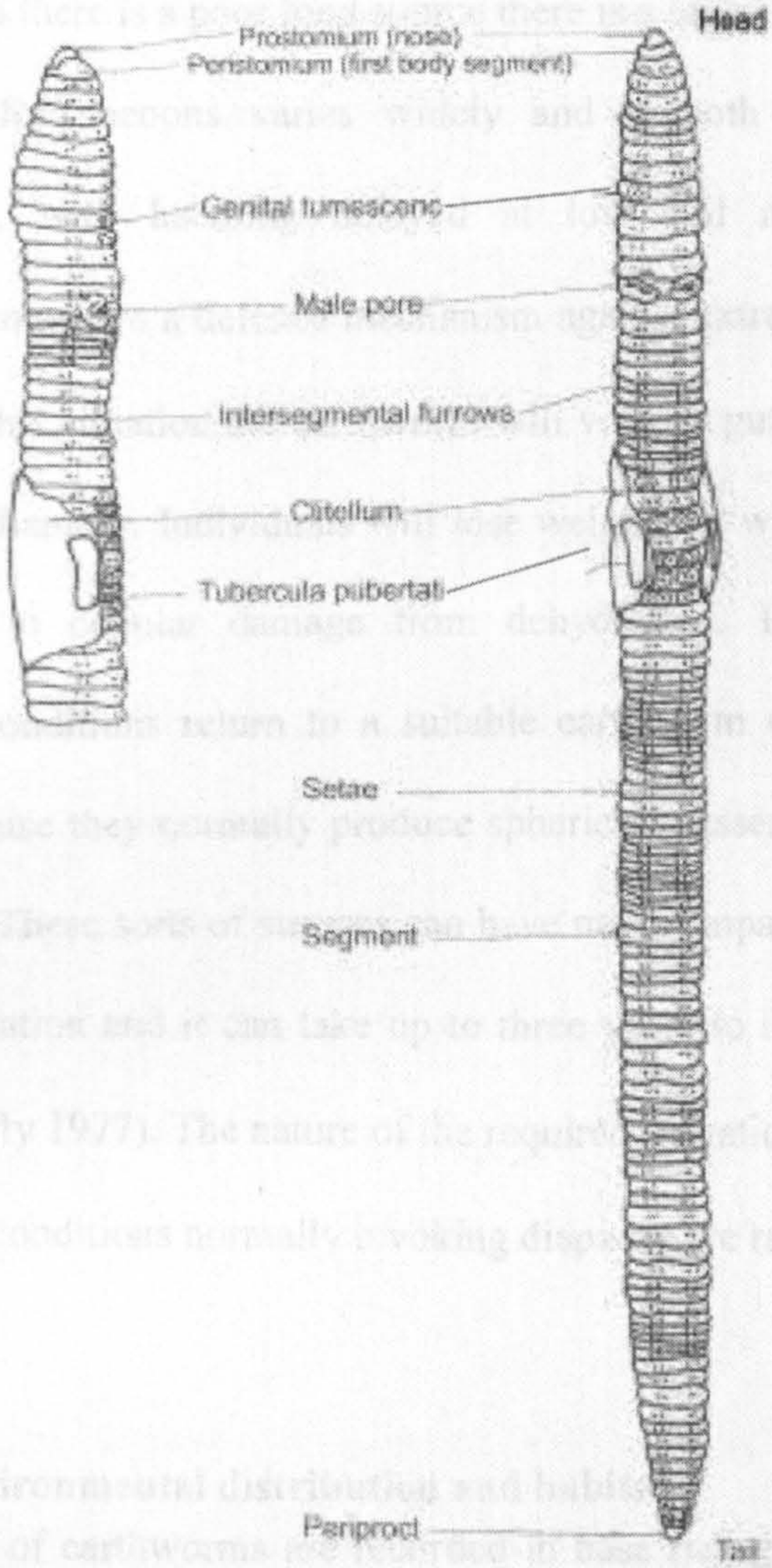


Figure 1.3: Stylised longitudinal plan of oligocheate worm showing common external features. Not drawn to scale. (adapted from Sims and Gerard 1999)

Earthworms are hermaphroditic and semi-continuous breeders but cannot self-fertilize (Brusca and Brusca 1990). Reproduction between an adult pair will result in the formation of a cocoon, which can be produced at any time of the year but activity normally peaks in late spring or autumn in the northern hemisphere. A single *L. terrestris* can produce up to 100 cocoons each year, generally hatching a single worm each (Lee 1985). The rate of reproduction is linked to the quality of the food available

to the earthworms; when there is a poor food source there is a lower rate of reproduction. The gestation period for cocoons varies widely and is both soil moisture and temperature dependent, with hatching delayed at low soil moisture or higher temperatures. Earthworms have a defence mechanism against extremes of temperature known as diapause. In this situation the earthworm will void its gut and surround itself with mucus in a lined chamber. Individuals will lose weight but will not suffer any of the problems relating to cellular damage from dehydration. This phase will be terminated when soil conditions return to a suitable earthworm environment. When earthworms leave diapause they normally produce spherical masses, similar to casts at the surface (Lee 1985). These sorts of stresses can have major impacts on the fecundity of the earthworm population and it can take up to three years to recover to pre-stress levels (Edwards and Lofty 1977). The nature of the required irrigation patterns on sports turf means that the arid conditions normally invoking diapause are rarely reached (Kirby and Baker 1995).

1. 2. 3. Earthworm environmental distribution and habitats

The largest populations of earthworms are recorded in base rich grassland soils under cool temperate conditions. Abundance, as with many studies in ecology is governed by the supply of food available to the earthworm, although factors such as disturbance often play a key role. Their distribution is therefore generally governed by the management strategy of the land they occupy (Muldowney *et al.* 2003). It is suggested that in the sports turf environment, the horizontal distribution of earthworms varies across a range of physiochemical gradients, these include pH, inorganic salts and particle size/soil texture (Kirby and Baker 1995).

Earthworm distribution is affected by compaction and bulk density of the soil: the more compact the soil the smaller the earthworm population. When earthworms are removed from the soil system (e.g. soil is treated with a vermicide) it will become more compact as soil pore space are not altered by the burrowing action of the worms (Boag *et al.* 1997). This makes a more adverse environment for the earthworms, thus reducing their abundance. Earthworms play an important part in maintaining percolation and infiltration in soils and thus the absence of earthworms has a major impact on the rate of drainage (Smettem 1992). The burrowing and soil mixing of earthworms results in the formation of water stable aggregates as well as aerating the soil. This results in an increased soil water holding capacity. Quantitatively it has been shown that earthworm worked soil has a gas volume that is increased by between 8 and 30%, and that the soil volume will drain between four and ten times faster than soil not worked by earthworms (Edwards and Lofty 1977).

Earthworms feed on organic detritus and undigested food material is then incorporated into the soil on egestion. There is evidence that soil fungi and bacteria provide an important food source to earthworms (Bonkowski *et al.* 2000). The current understanding of the interactions between earthworms and soil microflora is that soil bacteria have very little effect on feeding selection by earthworms. However, metabolites produced by soil fungi may provide a feeding cue in soil to the earthworms (Lee 1985). Interestingly, the same experiments have shown that earthworms cannot survive by feeding on fungi alone. This indicates that the system interactions are controlled by a large number of variables (Bonkowski *et al.* 2000). Experiments have shown that some species of bacteria and fungi are resistant to decomposition in

earthworm guts (Lee 1985). No single species of microflora is found exclusively in earthworm guts, but some evidence shows that there is a symbiotic relationship between the earthworms and soil bacteria (Lee 1985). Analysis using direct counts and 16S rRNA gene clone libraries show that microbial communities found in earthworm guts are dominated by a small number of phylotypes and associations are more opportunistic than obligate (Singleton *et al.* 2003). These findings have been reinforced by isolation of microbial community using phospholipid fatty acid analysis (PLFA) from bulk soil and soil found in the gut of earthworms, which indicate that distinct microbial communities are found in these two places (Sampedro *et al.* 2006).

Earthworm distribution and populations can be linked to the supply of their substrate. In ecosystems, earthworms are typically only responsible for a small amount of the energy transfer. In a temperate woodland *L. terrestris* consumes about 10% of the annual leaf litter fall with soil fungi and bacteria therefore decomposing the majority of the litter. The role of earthworms in the carbon and nitrogen cycle is not clear but the C:N ratio of their food source and surrounding soil will have an effect on the rate of growth and abundance of earthworms (Lee 1985). Where there is a high C:N ratio there will be a reduction in the number of worms found. The formation of micro and macro aggregates, and so to some extent soil structure and carbon cycling, is also dependent on earthworms. In an experiment where *Aporrectodea caliginosa* (Savigny) were introduced into soils, the formation of microaggregates in a soil that had previously been homogenised to <250 μm was enhanced within 12 days (Bossuyt *et al.* 2004). All anecic earthworms cause a significant difference in the pore structure through

burrowing and casting. This also has an effect on carbon mineralization and other biogeochemical cycles in the soil (Gorres *et al.* 2001).

The texture of the soil will also have an effect on the distribution of earthworms. The factor governing this is primarily the proportion of clay and sand particles within the matrix. Experimentally it has been shown that a greater number of *Ap. trapezoides* (Duges) are found in soils that are clay soils than sandy clay loams (Baker *et al.* 1998). The feeding of the earthworms is also selective relative to the soil particle size. Where possible the worms will avoid larger sand particles. This can be seen in a size particle analysis of earthworm casts that will typically contain less sand that the surrounding soil (Curry 1998). The earthworm's preference is for organic matter but the soil particles will be graded through the gut as a by-product (Pilar Ruiz *et al.* 2006). This action has an effect on the soil structure. Soil mixing in the gut with mucus, microbial exudes and fungal hyphae will bind particles together. Whether this egestion from the gut takes place into or onto the soil profile, it will still have a significant effect on pedogenesis.

The feeding habits of earthworms mean that they can re-distribute seeds within the soil matrix. Anecic worms can transport seeds to the surface via casting where subsequently they can be stimulated to germinate when they receive suitable moisture and light cues. The casts are also a good environment for seed germination. The soil particles are loosely packed allowing new roots to penetrate with greater ease: the casts are also high in available nitrogen (Decaens *et al.* 2003). In a controlled turf environment where fine leaved species of grass are used this can cause a significant problem with weed species. Invasion by *Poa* spp (especially *Poa annua*), and other weed species can be increased

by this sort of seed movement. Even if seeds are not transported to the surface by the worm then the cast at the surface of the turf still provides an ideal seed bed for wind distributed seeds (Decaens *et al.* 2003). Earthworms however can play a useful role in reducing thatch and increasing turf growth. In pasture land their presence is generally considered highly beneficial. Soil gut extracts from *L. terrestris* have been shown to contain metabolites that stimulate grass growth, such as the plant growth regulator indolyl-3-acetic acid (IAA). Other earthworm species have been associated with the secretions of other plant growth regulators (Lee 1985).

1. 2. 4. Earthworm interactions with the environment

On pasture land earthworm density is normally around 200 m^{-2} to unreported depths. However the engineered environment of sports turf means that this density is considerably reduced (Stewart 1994; Binns *et al.* 1999). Only relatively few of the 25 species of earthworm in the UK have any major beneficial effects in relation to the incorporation of organic residues in the soil and increasing drainage under grasslands (Stewart 1994).

The vertical distribution of earthworms also varies considerably with the time of year. The species distribution in the UK during spring months varies to a range of depths (Table 1.1):

Table 1:1: Position in the soil profile of frequently found worm species in pastureland (adapted from Edwards and Lofty 1977).

Depth in soil profile	Species commonly found
Surface organic horizon	*Dendrobaena octaedra*
Top 8 cm of soil	*Allolobophora cholorotica*
	Aporrectodea caliginosa
	Aporrectodea longa
	Aporrectodea nocturna
	Aporrectodea rosea
	Lumbricus castaneus
	Lumbricus rubellus
	Lumbricus terrestris
	Octolasion cyaneum (immature)
	Octolasion tyrtaeum tyrtarem (immature)
Top 15 cm of soil	All mature worms present
Depths of up to 45 cm	*Aporrectodea longa*
	Aporrectodea noctura
Dépths of up to 250 cm	*Lumbricus terrestris*

The pH of the soil has been demonstrated as an important property modulating earthworm distribution. Baker and Whitby (2003) demonstrated that earthworms showed an aversion to soils of pH less than 4.5, but only a slight aversion to soils with a pH of above 8. No trend could be seen between these values. The control of worms on sports turf has attempted to take advantage of this knowledge. By applying a sulphur-based fertilizer, with a low pH the soil is acidified; this practice is common as it results in soil conditions that are hostile to fungal diseases, such as *Microdochium nivale*, and so is commonly practiced. The disadvantage of this is that it produces a root zone environment that is less beneficial for the growth of the turf. Other workers have concluded that *L. terrestris* can tolerate a wide range of soil pH, but that they will die in soils with a pH of less than 4.4. For most other species the minimum pH range is between 5.0 and 6.0 (Edwards and Lofty 1977).

There are two different kinds of earthworm burrows, permanent burrows: that remain open to the surface (from anecic species) and temporary burrows that are back-filled by the earthworm (from endogeic species). These different burrow types have different effects on the soil. The strength of the walls of these burrows is dependent on the hydrostatic pressure that the earthworm can generate in the coelomic cavity. This will have an effect on the stability and drainage capacity of the soil. The biggest physical effects of the burrows on the soil are mostly a function of pore space both at the macro and micro scale, increasing aeration and drainage. Statistically, it is difficult to correlate soil porosity with earthworm biomass (Lee 1985). It is suggested that the presence of greater pore space is not the most important factor but the movement and relocation of pore spaces through the connection of macropores and thus creating by-pass flow that is the critical factor for increasing drainage and flow rate. However, Smettem (1992) developed a mathematical model to describe the relationship between earthworms and soil hydraulic properties. This showed that burrow length and surface area of the burrow opening were the most critical factors in increasing flow rate. Despite the range of views on the mechanism of effects on drainage, data on the physical effects cannot be denied: burrows increase the soil air volume and increase drainage rates (Edwards and Lofty 1977).

The spatial habitat of earthworms is highly complex with both inter- and intra- species interactions having an effect on the rate and nature of activity. Capowiez (2000) studied the behaviour of *A. noctura* and *A. chlorotica* in relation to each other. Both of these species are known to cast at the surface. This study showed that the number of individuals and species distribution within the microcosm reduced the casting behaviour

of *A. chlorotica*, while *A. noctura* often produced surface casts irrespective of the species distribution in the microcosm. However, these experiments also showed that the surface exploration of these earthworm species is dependent on population size and activity of the other species present however; *A. noctura* will always investigate the largest surface area.

1. 2. 5. Earthworms in sports turf

The ecology of oligochaeta pest species, unlike that of other invertebrate classes such as Insecta, is still under-represented in research work. Many studies are still descriptive and correlative, focussing on the environments that worms inhabit (Curry 1998). There are no time-based studies of effect, either beneficial or deleterious, of earthworms on sports turf.

On the playing surface where ball roll (e.g. golf and bowls) or bounce (e.g. cricket and tennis) is important earthworm casts can be problematic because they can result in an uneven playing surface (Stewart 1994; Binns *et al.* 1999; Baker *et al.* 2000) but the problems extend beyond this. The surface earthworm casts present problems with maintenance of the sward, especially with mowing. Most high performance grass surfaces are cut very close to the ground. Mowers are typically set to a height of 5 mm or below on golf greens (Baker and Binns 1998). Earthworm casts, which can be up to 25 mm in height (Lee 1985), will stand proud of the grass and may damage and blunt mower blades. The earthworm casts can also be smeared on the surface, which can result in problems with drainage. Smeared casts will also damage and prevent the fine-leaved grass species used for sports turfs from growing (Hope 1990). In many sporting

situations, the cosmetic appearance of the turf is an important attribute, and earthworm casts are considered unsightly (Stewart 1994).

In the past the problems presented by earthworms have been overcome by killing all earthworms present. This was achieved using surface applications of organochloride chemicals that have now been banned due to their inherent toxicity and effects on non-target organisms. The practice of earthworm eradication in sports turf has a major impact on soil compaction, increasing it far above that found in a natural system. This has a knock-on effect on the drainage because there is less penetration of water into the soil. This results in a reduction in both nutrient cycling and plant growth (Hope 1990; Stewart 1994). In the absence of earthworms, sub-surface problems such as thatch formation occur. This can have major impacts on the health of the turf and impact on the overall sustainability of the sports surface.

Groundsmen around the world have for years been trying to strike a balance between the positive and negative effect of an earthworm population in the turf environment. They have sought to balance the increases in aeration, drainage and nutrient cycling provided by earthworm populations and their deleterious cosmetic effects (Baker *et al.* 1995; Perris 1996a). Until 1992 the most effective solution to these problems was earthworm eradication through use of chlordane (Figure 1.4). This is a highly chlorinated chemical, toxic to a wide range of organisms. However a change to the Control of Pesticides Regulations 1986, revoked its Application Certification on the 31st December 1992 because of it toxicity to other, non-target organisms (Baldwin and Bennett 1990; Perris 1996b; Cook *et al.* 1997).

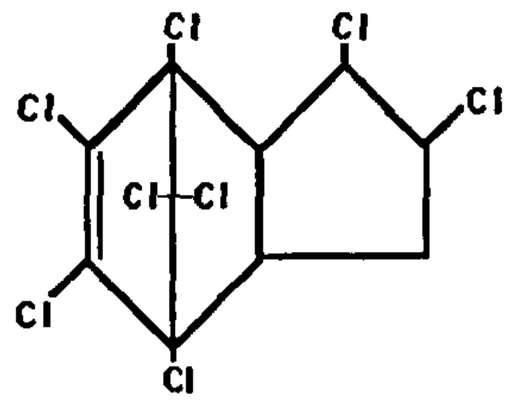


Figure 1.4: Structural formula for chlordane

The dominantly problematic species within sports turf have been identified as *Ap. longa*, *Ap. caliginous* and *L. terrestris* (Jefferson 1956), but the interaction with the environment has been poorly understood as most work has focussed on killing or deterring all worms (Woolhouse and Wright 1984; Cook *et al.* 1997; Baker *et al.* 1998). Data collected in relation to golf courses is largely qualitative, but indicates that most earthworm-related problems are observed on tees and fairways rather than greens. It also suggests that problems are more prevalent on golf courses that have been developed from agricultural land than on parkland or links courses (Baker and Binns 1998). This probably reflects the long-term damage to the earthworm population by persistent use of vermicidal chemicals, and the slow recovery period observed with earthworms (Edwards and Lofty 1977).

1. 2. 6. Golf course playability

The playability of a golf course can be considered in terms of the degree to which a round is enjoyable to play as a game and how aesthetically pleasing the course is to walk. This contributes significantly to the overall quality of a single round. For this reason it must be a subjective matter, one person's opinion of an interesting round of golf may not be the same as the next, just as two people may disagree over the aesthetics of a course layout. For this reason different course management techniques,

and management teams, will result in different levels of playability on any course. This coupled with the fact that different standards of golfer command different requirements from a course means that many factors with each golf courses playability must involve compromises. These factors can be structured into the physical, biological and aesthetic component.

Physical design of any golf course has a major influence on the overall playability of a course. The layout and the way the course is set up for play has major effects on the way that individuals perceive the course. Golf courses are normally described as either penal or strategic. Penal courses normally date from 18th century and are harsh in design with a great number of hazards such as bunkers or water features. Strategic courses are a design compromise, options for simple shots can be made for less proficient players to complete the course, but the more challenging shots are rewarded for the more technically proficient. When golf courses are being laid out these options are always present and must be addressed; should the game be oriented to playing the longest shot possible, or should it be for greater technical accuracy.

The biology of a golf course impacts the playability which results from an interaction of many factors. The quality of the turf sward has a major impact and components such as smoothness, texture, uniformity in growth (including growth density), colour of the plants and presence/absence of weed species. The mowing of each area of the course can affect both the skill required to play golf and the perception of the surface. The amount of water that is applied can radically alter the playing surface (Balogh and Walker 1992), affecting both ball bounce and ball roll. Other biological features, such as

trees, can have major impacts on golf courses playability, affecting both grass growth and shot selection.

The aesthetics of golf courses is a highly subjective matter, being a composition of both the biological, physical and psychological factors. Assessments of the aesthetics with respect to playability of each course must be made on its own merits. In terms of presentation it is unfair to compare one course at which club standard golf is played to one which regularly hosts national and international competitions. Earthworms also play a major role in the aesthetic perception of a golf course, through surface casting.

When considering the playability of a course all of the above factors must be taken into account. Currently no clear hierarchical structure has been elucidated: no one factor has been identified as being more important than any other. With a poor physical structure to the course (i.e. a hard penal course), but perfectly conditioned turf the game would be too challenging, but a course with 18 straight holes with no hazards is likely to be considered boring. Biological components at the whole course scale must be balanced in terms of cultivated grasslands blending into the surrounding landscape. At the individual hole level the turf must be of acceptable quality for play of the individual golfer. The playability of a golf course is as much dependent on the physical set up of the course as it is in the mind of the player and as such can only ever be measured qualitatively.

1. 2. 7. Anthropogenic soil materials and golf course soil profiles

The different management and construction strategies for the surfaces found on golf courses also affects earthworm distribution and general soil ecology. Greens are

regularly mowed to a short height (approximately 5 mm) – during the growing season, on a daily basis. Fairways however are mowed to longer lengths and less frequently, providing a more suitable environment for earthworms. The different surface construction techniques may also play a part in altering population distributions (Figure 1.5). International standard greens are typically constructed to the United States Golf Association (USGA) specifications where the root zone material is highly prescribed and tightly regulated (Table 1.2). The USGA specified a rootzone was designed to be a free draining surface with a large number of macropores, even in the absence of earthworms. This design ensures that greens rarely become waterlogged, even after intensive periods of rain (USGA Green Section Staff, 2004).

Table 1:2: Specification of the particle composition of rootzone used for USGA design greens (USGA Green Section Staff 2004).

Particle Type	Particle size	Composition by weight
Fine gravel	2.0 – 3.4 mm	Not more than 10% of the total particles in
Very coarse sand	1.0 – 2.0 mm	this range including a maximum of 3% fine gravel (preferably none)
Coarse sand	0.5 – 1.0 mm	Minimum of 60% of the particles must fall
Medium sand	0.25 – 0.5 mm	in this range
Fine sand	0.15 – 0.25 mm	Not more than 20% of the particles may fall within this range
Very fine sand	0.05 – 0.15 mm	Not more than 5%
Silt	0.002 – 0.05 mm	Not more than 5%
Clay	Less than 0.002 mm	Not more than 3 %
Total fines	Very fine sand and silt and clay	Less than or equal to 10%

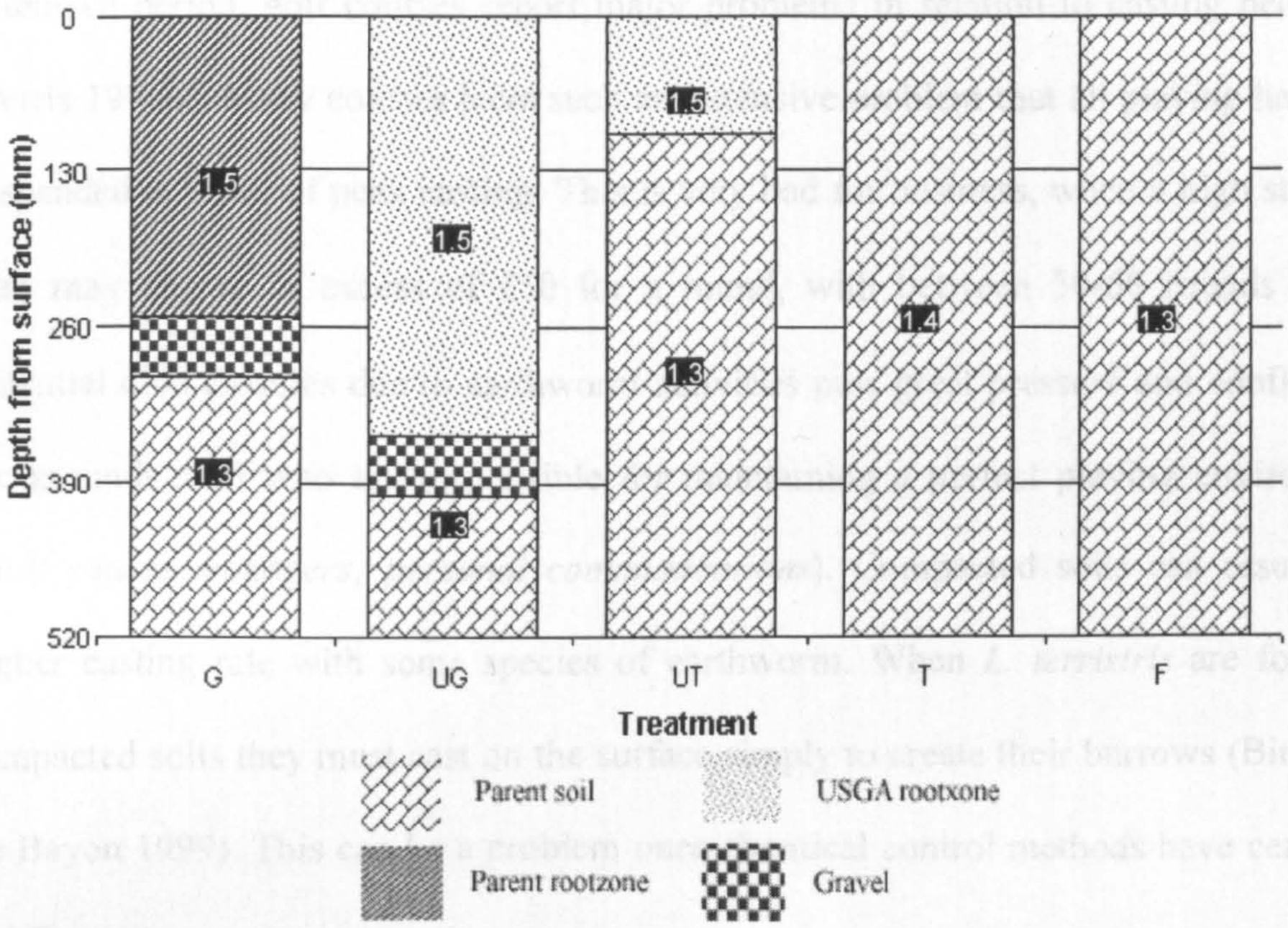


Figure 1.5: Different anthropogenic soil profiles found on golf courses and associated mean dry bulk densities (g cm^{-3}) denoted in black text boxes to which construction material is commonly packed with respect to Standard greens (G); USGA greens (UG); USGA topped tees (UT); Standard tees (T) and Fairways (F).

1. 2. 8. Working with, or against, earthworms in sports turf

There are links between earthworm populations and drainage in winter sports surfaces (e.g. rugby and football pitches). Here, the aesthetic problems caused by earthworms casting on the surface are considerably smaller than the advantages of increased drainage and mass transfer known to be associated with soils that have large, active earthworm communities. Total control leads to greater problems of thatch build up and compaction that in turn lead to ponding and turf/surface degradation. On football pitches that do not have a worm control policy there are fewer problems with localised drainage (Baker 1981). On professional football pitches control is frequently applied because of cosmetic considerations, generated through economic concerns (Raikes *et al.* 1994). In Germany, where chemical control of earthworms has been forbidden for an

extensive period, golf courses report major problems in relation to casting behaviour (Perris 1996b). Many courses have such an extensive problem that all playing has to be suspended at times of peak casting. This is very bad for business, when a high standard club may charge in excess of £50 for a round, with between 50-60 rounds a day. Potential club closures due to earthworm activities puts great pressure and conflicts on the grounds staff who are responsible for maintaining a perfect playing environment (*Golf course managers; personal communications*). Compacted soils can result in a higher casting rate with some species of earthworm. When *L. terristris* are found in compacted soils they must cast on the surface simply to create their burrows (Binet and Le Bayon 1999). This can be a problem once chemical control methods have ceased to be effective.

Earthworm problems are also recorded on grass tennis courts, but on these surfaces where accurate ball bounce is essential the behaviour has been studied in greater detail. The greatest intensity of casting behaviour is seen during the spring and autumn, when the soil moisture content is optimal for the earthworm. The fact that tennis is principally a summer sport means that cultural techniques (such as clipping removal and the use of acidic fertilisers) can be used to reduce the problem. For this reason many tennis clubs choose not to employ a chemical vermicidal regime.

Further advantages of the presence of earthworms in sports turf have been considered by Potter *et al.* (1990) in a study of thatch degradation. They noted that it is essential for the health of the turf that thatch is broken down and the nutrient cycling within the soil system is maintained. Earthworms play an important role in this. Their evidence

indicates that earthworms do not derive any calorific value directly from the thatch that they ingest. The earthworm's role in thatch breakdown is to re-distribute it within the soil profile, exposing it to microbial and fungal soil biota that is capable of decomposing it. The net result is an increase in earthworm available nutrients (the soil fungi and bacteria) and a reduction in thatch build up. Other work also indicates that earthworms are instrumental in breaking down thatch showing a simple relationship where the use of organochlorides (such as chlordane) increases thatch build up (Randell *et al.* 1972).

1. 2. 9. Control methods for earthworms in sports turfs

The banning of organochloride based chemicals for the control of earthworms sparked an increase in the research into the problem of earthworm control. In the UK, this work has principally been carried out at the Sports Turf Research Institute, Bingley, UK. A range of cultural and chemical controls have been considered.

Attempts were made to control the casting of earthworms before the development of chemical control methods. Both Dawson (1938) and Baker *et al.* (2000) showed that the use of acidifying fertilisers and the boxing and removing of grass clippings could reduce the number of earthworm casts formed. On small trial plots this kind of treatment showed a reduction to as low as 4 casts m^{-2}, when the grass was cut to a standard 13 mm (Baker *et al.* 2000). There are considerable logistic considerations to implement this as a management strategy *vis a vis* the disposal of green compost once produced. The disposal of such a large amount of green waste would cause logistical problems. This kind of management strategy would therefore have to be reserved for the most sensitive areas on a golf course. With such an aggressive break in the nutrient cycling within the

soils system the long-term impacts on fertility and requirement of inorganic fertilisation needs to be addressed in relation to the sustainability of sports facilities (Baker *et al.* 1996). The use of chemicals such as sulphur and aluminium sulphate can result in a rapid decrease in the pH of the surface horizons of the soil to a depth of approximately 150 mm (Baker *et al.* 1996). While these chemicals can be shown to suppress earthworm populations the lowering of the soil pH also produces a suboptimal growth environment for the turf. An increase in moss, and in some cases damage to the grass leaves (tip burn), has been recorded with acidic fertilisers (Kirby and Baker 1995; Baker *et al.* 1996).

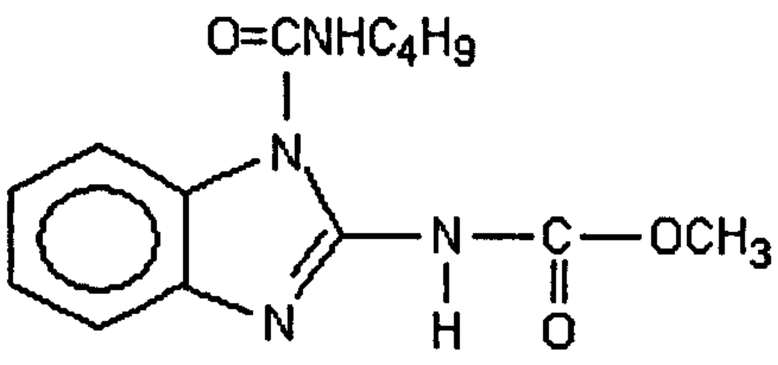
Prior to the widespread use of chlordane, earthworms were controlled by expellant methods. This method relies on the behavioural physiology of earthworms to move away from harmful substances, i.e. to the surface (Hope 1990). A range of control expellant materials have been trialled or reported (Cook *et al.* 1997; Baker and Binns 1998) but the most popular, in order of effectiveness are formaldhyde, mustard powder, potassium permanganate, Rotenone[3] and detergent. None of these materials produced a long term control, i.e. longer than several months (Cook *et al.* 1997). A further consideration must be made about this method of controlling earthworms relating to its cost effectiveness. Some reports suggest that approximately 100-150 individual earthworms can be present per m^{-2} on a golf fairway (Randell *et al.* 1972). An expellant that resulted in even 50% of this number at the surface would cause problems with 'harvesting' of earthworms and their disposal. It is conceivable that there could be a

[3] Rotenone is an organic pesticide derived from the plants in the genus *Lonchocarpus* (Pesticide News 2001)

resale market for these earthworms as either feed meal to poultry, for vermiculture, or retail as fishing bait.

Green keepers have long been aware that chemicals not specifically listed to control earthworms will reduce the populations under their sports fields (Woolhouse and Wright 1984). For example, chemicals listed as fungicides, such as Benomyl, Carbendazium, Thiabendazole and Thiophanate-methyl (Figure 1.6 a-d) will all have an effect, albeit variable, on earthworms. Woolhouse & Wright (1984) concluded that Thiabendazole was the least effective at controlling earthworm populations compared to a control where no biocides were applied. Thiophanate-methyl was shown to cause the greatest reduction in earthworm casts, but only by 25% compared to the control. This implies either a tolerance in some species or that the most effective concentration as a vermicidal agent for these fungicides would produce toxic effects to either the grass or other components of the biota.

(a)



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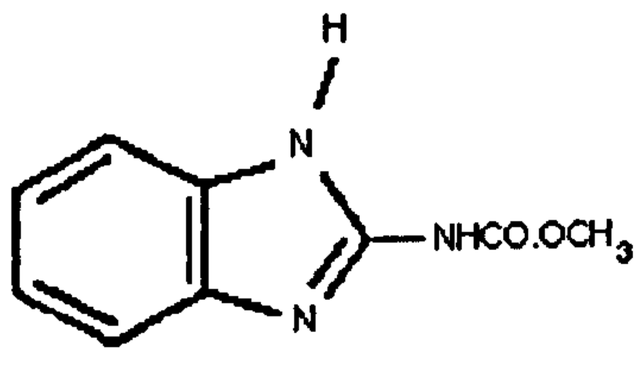

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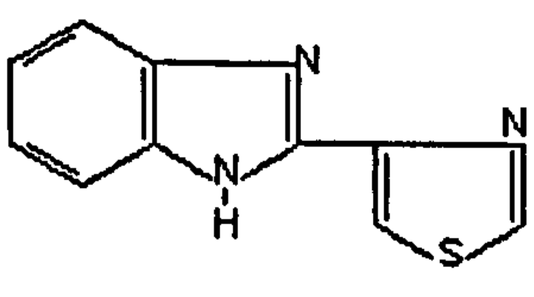

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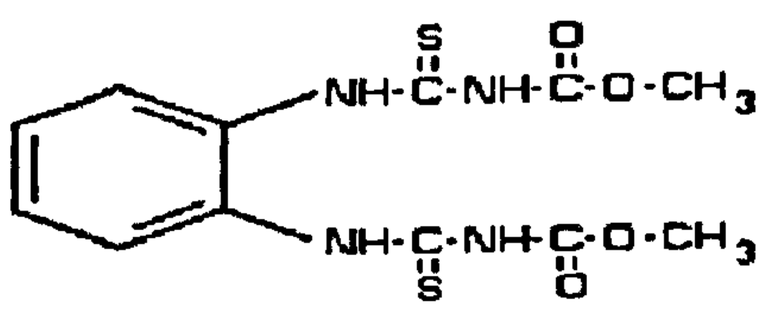

Figure 1.6: Structural formulas for biocidal chemicals currently used on sports turf. a) Benomyl, b) Carbendazium, c) Thiabendazole, d) Thiophanate-methyl.

The control duration for earthworms using these chemicals is considerably shorter than with chlordane, typically in that they are effective for months rather than years, between 5-18 months depending on soil and environmental conditions (Baker *et al.* 1998). Problems have also been reported with phytotoxicity for some of these active ingredients. The structural formula of these chemicals (Figure 1.6) also suggests they are less toxic than chlordane (Figure 1.4). The mode of action of all these vermicides are considerably different to that of chlordane, working by steric hindrance of enzymatic processes rather than the liberation of chlorine free radicals which then destroy cellular structure.

It has previously been assumed that the high proportion of sand in this matrix means that earthworms are disinclined to feed and so colonise these areas of a golf course due to the sands abrasive properties. A trial control method on standard greens at the Sports Turf Research Institute (Bingley, UK) has been to top-dress greens with angular sands to create a hostile an environment for the earthworms. However, as with many methods it produces an environment that is suboptimal for turf growth as the more sandy soil retains less nutrients (Cook *et al.* 1997; Baker *et al.* 2000).

Work has been carried out using crushed coal slag (3.2 mm diameter) in the USA (Williamson 2004) and although good control results were initially seen there are potential considerations with the long term effects of the treatment. When compared to chemical controls however, this method is not considered effective (Williamson and Hong 2005).

A range of physical methods of earthworm control have also been investigated (Kirby and Baker 1995). These include electrical expellance methods and biological control. Both of these methods have flaws or practical problems. Expellant methods using an electrical current would force large numbers of worms to the surface creating collection problems. It has also been demonstrated that earthworms can migrate downwards to avoid the electrical current (Rushton and Luff 1984; Kirby and Baker 1995). A further problem with this method is that the conductivity of the soil is moisture dependent. Where there is a greater soil moisture content the sphere of influence is increased. This produces a very wide range of control efficiencies. An effective biological control would be an ideal solution to the problem (Murdoch *et al.* 1985). The release of a specific parasite for anecic earthworms could minimise groundsman's problems relatively quickly. A species that could accomplish this is *Artioposthia triangulata* (Dendy) - New Zealand flatworm. This species preys on *Lumbricus sp.* and is capable of considerably changing earthworm population dynamics (Boag *et al.* 1997; Blackshaw 1997). However there are some major drawbacks with this approach, principally arising because earthworms in agricultural and all other ecological systems are considered highly beneficial. An escape of the control vector into the wider environment could have undesirable consequences. *A. triangulata* has caused significant, and deleterious changes to the earthworm populations in Northern Ireland where it is found as an invasive species (Muldowney *et al.* 2003). Its territorial range and life cycle means that it would be inappropriate to release as a biocontrol agent.

1. 3. Experimental approach and directions for research

From this review it is clear where there are gaps in understanding and knowledge. This thesis therefore has two aims:

A. **Investigation of the ecology of earthworms on sports turf.**

B. **The control of earthworms on selected surfaces of sports turf.**

Golf courses will be investigated in relation to these problems, with the potential that they may be extrapolated to the wider arena of sports facility management. Interpretation of how each investigation informed understanding, and its potential application with regards to the control of earthworm casting is given in Chapter 10.

- **Earthworm species abundance, diversity and activity – Chapters 3, 4 and 5.**

  Population distribution and population density studies have been carried out for arable and grasslands (Edwards and Lofty 1977; Buck *et al.* 2000; Gorres *et al.* 2001; Nuutinen *et al.* 2001) but cannot be extended to the highly engineered anthropogenic soil profiles found on golf courses. This study elucidated links between earthworm activity and local environmental conditions.

  A comprehensive and quantitative assessment of the scale of the problem of earthworms casting on the three most important surfaces on a golf course: the tees, fairways and greens. Some research has indicated which earthworm species cause casting problems in sports turfs (Baker *et al.* 1995; Cook *et al.* 1997; Baker and Binns 1998; Binns *et al.* 1999; Baker *et al.* 2000). However, much of this work is qualitative and lacks investigation of mechanisms. An increase in knowledge here would be beneficial to aid the targeting of research into environmentally sustainable controls of earthworm casting.

- **Effects of the physicochemical parameters of the soil on earthworms – Chapters 3 and 8.**

Qualitative evidence suggests that casting (and so by analogy, earthworm population) is lower on greens of golf courses compared to tees and fairways (Baker and Binns 1998). A controlled experimental system was used to determined whether the construction profile of the soil was affecting the earthworm distribution. Quantitative information of this nature can be used to inform decisions on how to physically manipulate the environment to reduce earthworm casting.

- **The relationship between earthworms and the size and structure of the soil microbial community – Chapters 6 and 7.**

  The soil microbial community is intrinsically linked to the earthworm community size and structure through its function in providing food to the earthworm (Lee 1985). An investigation of the relationship between the soil microbial community and the anthropogenic soil materials of golf courses was used to increase knowledge of the causal relationships manifest in earthworm casting behaviour.

- **The mitigation of earthworm casts – Chapter 9.**

  A number of control mechanisms to reduce or eliminate earthworm populations in the most aesthetically sensitive surfaces of golf courses required consideration. Investigations into cast mitigation on tees and USGA greens were made. This worked on the principle that any control method developed must be reproducible, and have potential to be scaled up to whole golf course applications.

Chapter 2: Generic methods and site locations

A range of standard methods are used to describe both the physicochemical and biological nature of soil. Physicochemical parameters such as soil pH, cation exchange capacity, soil organic matter (loss on ignition), particle size analysis, total organic and inorganic carbon content and total nitrogen content can be used to describe the physical and chemical environment with which the soil biota interact. At the microbial scale measurements of community structure (using phospholipid biomarkers) and community size (using estimates of soil biomass carbon) give us insight into the causal mechanisms operating in the environments that are being studied. Both physical and micro biological parameters are intrinsically linked and combine to characterise the soil habitat in which earthworms live, and in turn modify.

It is impossible to fully quantify this habitat, however, the measurements described in this chapter were chosen as previous research has shown that they have the most significant effect on earthworm distribution and abundance (Satchell 1967; Edwards 1996; Kretzschmar 1998; Lavelle 2001; Baker and Whitby 2003).

2. 1. Total cation exchange capacity

2. 1. 1. Theory

The determination of cation exchange capacity (CEC) gives an overall measure of the soils total ability to exchange and retain cations. Lee (1985) suggests that CEC is more important than pH in determining size and structure of earthworm community due to its implied measure of soil fertility. Fertile soils, high in organic matter, have a high CEC and are capable of supporting larger and more varied earthworm populations. Within one soil textural class the potential range of CEC is wide, therefore, CEC must be

determined for samples that vary spatially (Table 2.1). The CEC of a soil is dependent on the pH, soils with higher pH generally have a higher CEC due to an increasing availability of sites for exchange because of the reduction of sites occupied by H_3O^+ ions. For this reason CEC is measured at a controlled pH (FitzPatrick 1980).

Table 2.1: Approximate ranges of CEC for five different soil types, measured by Ba^{2+} substitution and exchange with Mg^{2+} (after Bascomb 1964)

Soil type	Usual CEC range (centimol kg^{-1})
Peat	10 to 30
Sandy loam	3 to 17
Silt loam	10 to 25
Clay loam	4 to 30
Clay	5 to 60

The method used makes two assumptions:

1. There is no steric hinderance within the system, Ba^{2+} ions can physically reach all sites on which they can potentially exchange with other ions, and
2. There are no other ions already bound to the soil matrix that have a greater electrostatic charge or atomic radius than Ba^{2+}. If this is the case then they will be bound tightly to the soil matrix and assessment of CEC by exchange of these ions will be impossible.

From this method it is not possible to determine the proportions of cations present in the soil. The typical order for ease of disassociation from the soil matrix is $Ca^{2+} > Mg^{2+} > K^+ > NH_4^+ \equiv Na^+$, from strongest to weakest associations. The determination of these ions proportions is only possible by repeated washing of soil with increasing atomic radius and charge cations and measuring the ionic concentration by flame atomic absorbance spectrometry.

2. 1. 2. Outline of method

This method was derived from ISO 11260:1994 (ISO 1994) and Bascomb (1964). A 5.00 g (< 2 mm) sub-sample of soil was base saturated with buffered Ba^{2+} (1:1 mix of triethanolamine: 1 M $BaCl_2 2H_2O$, adjusted to pH 8.1). Soil bound Ba^{2+} ions were then exchanged with Mg^{2+}, quantitatively measured at one decimal place by back titration with EDTA. Erichrome Black was used as an indicator buffered with 1 M NH_4OH in a ratio of 1:3, indicator to buffer. Results were reported in centimols kg^{-1} at one decimal place.

2. 2. Assessment of soil pH

2. 2. 1. Theory

pH is the measure of H_3O^+ ions that the soil will disassociate from the matrix when in an aqueous solution. Different soil particles are capable of forming buffers between the water and H_3O^+ ions in different ways, hence soil pH varies with soil textural class (Brady and Weil 1999). A soil's mineralogy will also impact on the pH: where the parent material is calcareous, the high proportion of carbonate minerals within the soil matrix results in a higher pH. Other parent materials which contain high proportions of free metals, such as aluminium or iron will lower the pH of the soil. In soils that are acidic the availability of certain elements (especially metals) increases. In these conditions a greater proportion of the soil microbial community is likely to be fungal. At high pH ammonia is more likely to be lost through volatilisation and the soil microbial community will be dominated by bacteria and actinomycetes (Harris 1989). For the majority of soil macro-organisms it is suggested that environmental pH is the most important factor in determining spatial distribution. Most soils pH ranges between pH 4 and 10, only at the extremes of this range are earthworms not recorded (Brusca and Brusca 1990).

In land management terms the pH of an alkaline soil can be lowered by adding sulphur, iron sulphate or aluminum sulphate (although this is generally expensive) or using urea phosphate, ammonium nitrate, ammonium phosphate or ammonium sulphate also lower soil pH. Within the golf industry it is uncommon for golf course managers to need to increase soil pH; however this can be achived by the application of lime.

2. 2. 2. Details of the method

A soil slurry of 1:2.5 soil to water was produced using air dried soil that was sieved to pass through a 2 mm sieve. This solution was allowed to stand for 16 hours then stirred before the pH was measured using a glass electrode pH meter (MAFF 1969; Gasser 1973).

2. 3. Assessment of soil organic matter by combustion

2. 3. 1. Theory

The amount of soil organic matter present is of particular importance as this is the fraction from which earthworms extract their calorific requirements (Lee 1985). The percentage organic matter in soil can be assessed by oxidation of all organic material in the soil. This is most simply achieved by ignition at 405°C. The assumption is made that all weight loss from the sample is attributed to the oxidation of organic matter. This 'loss on ignition' method is only reliable with soils that are non calcareous or do not contain fossilized carbon as these soil constituents will be lost as the soil matter is oxidised (FitzPatrick 1980). Where carbonate contents are high or the fraction of soil organic matter is low, the optimum method for calculation of soil organic matter is by wet oxidation of soil organic carbon and mathematical conversion to soil organic matter (Brady and Weil 1999).

2. 3. 2. Details of the Method

The weight change of approximately 10 g sub-sample of oven dried soil (mass recorded at four decimal places), sieved to pass through a 2 mm sieve was calculated after ignition in a muffle furnace at 405°C for 6 hours. The loss on ignition (*i*) is expressed as a percentage of the original oven dried mass of soil (*h*) and related directly to the organic matter content of the soil on a percentage mass basis (Equation 2.1).

$$\%\,\text{Loss on Ignition} = \left[\frac{i-h}{h}\right] * 100 \qquad (2.1)$$

2. 4. Assessment of particle size distribution in soil using hydrometry

2. 4. 1. Theory

Many of the physicochemical properties of soil relate to the size, and the distribution of sizes, of the mineral soil forming particles. These particles are commonly divided into: sand (2.00 mm to 0.063 mm), silt (0.063 mm to 0.002 mm) and clay (< 0.002 mm), which are used to draw textural classifications of the soil (Figure 2.1). Different textural classes have varying characteristics. Soils with a high proportion of clay size fraction will generally have high CEC and low hydraulic conductivity due to clay matrix exchange sites and small pore size distributions respectively. Soils with a high proportion of sand will have a low CEC (and consequently low nutrient retention), low buffering capacity due to the low chemical activity of silicate sand particles and high hydraulic conductivity as a consequence of larger pore diameters between particles.

The relative proportions of these fractions can be determined by hydrometry in a soil-water suspension of known soil mass (Bouyoucos 1951). Assuming that soil particles are spheres, and flow is laminar, the rate of settling of soil particles can be approximated from Stokes' law (Equation 2.2).

$$v = \frac{2}{9} g r^2 \frac{(\rho_1 - \rho_2)}{\eta} \qquad (2.2)$$

Where:

v = velocity (m s^{-1})
g = acceleration due to gravity (m s^{-2})
r = effective radius of particle (m)
ρ_1 = liquid density (kg m^{-3})
ρ_2 = particle density (kg m^{-3})
η = liquid viscosity (kg m^{-1} s^{-1})

It follows therefore, that the largest particles have the greatest settling velocities and settle first. By determination of the specific gravity of the soil at a specific time following stirring of the suspension, the specific gravity of a soil suspension will be proportional to the mass of the soil particles of a known maximum diameter in suspension. The settling velocity is a function of the fluid viscosity, which in turn, is a function of temperature, it is therefore necessary to record temperature at the point of measurement. Other methods have been developed to measure the particle size distribution of soils e.g. pipette method and laser sizing (Brady and Weil 1999). These methods are capable of reporting results with a greater accuracy (two decimal places), however this method (precise to two significant figures) was selected due to the cost and large number of samples requiring processing.

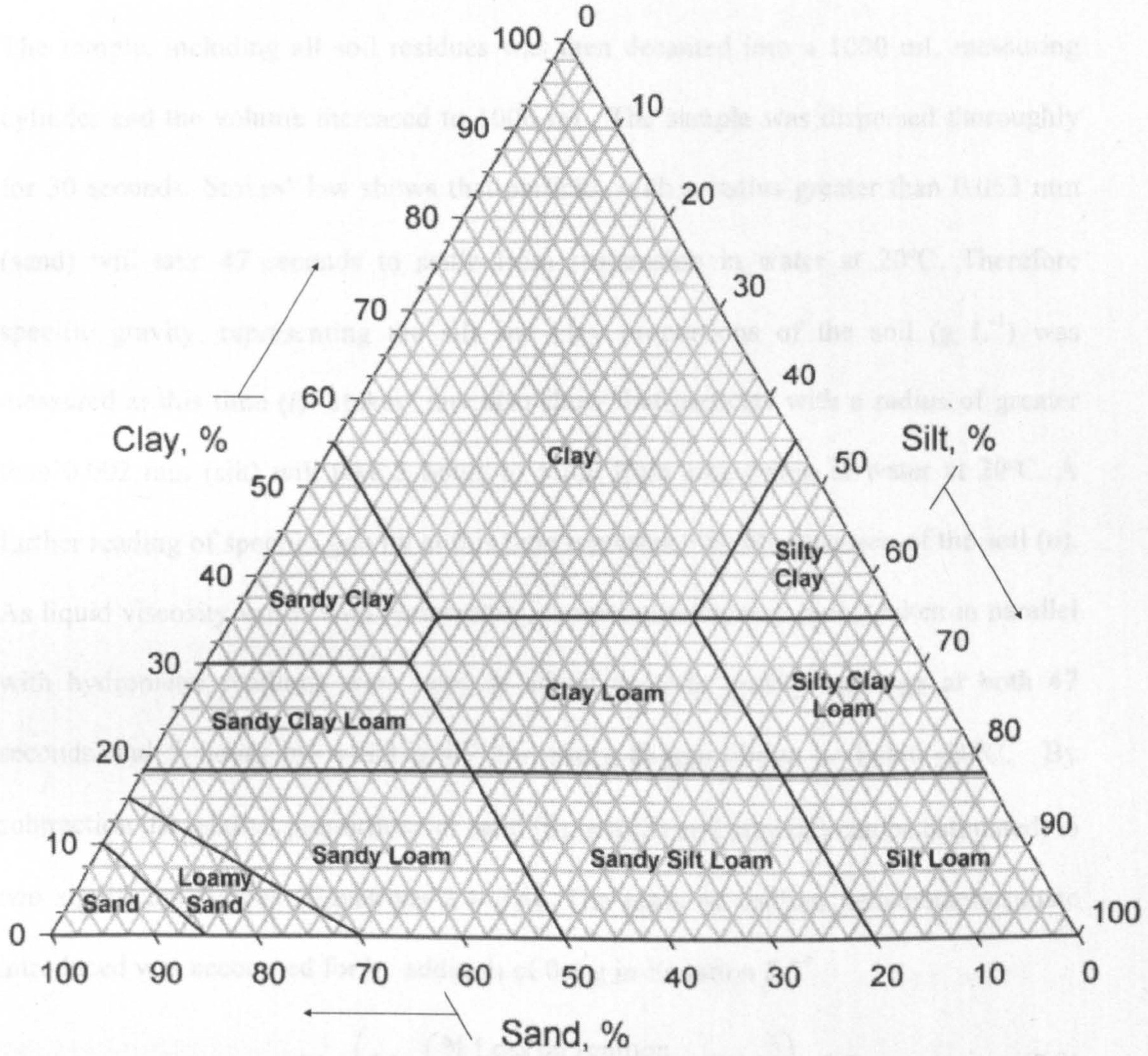

Figure 2.1: Textural triangle of England and Wales for mechanical analysis of soil (derived from Hodgson 1997)

2. 4. 2. Details of the method

This method was adapted from Harris (1989) and Bouyoucos (1951). Soil carbonates were destroyed using 1 M HCl then a 50.00g oven dry sub-sample was used for analysis. Soil organic matter was destroyed using 100 mL H_2O_2, applied in two equal aliquots. After 16 hours incubation the sample was boiled for 30 minutes. The volume was made up to 400 mL and then mechanically stirred for 30 minutes. A 10 mL aliquot of dispersing agent (sodium hexametaphosphate) was added.

The sample, including all soil residues was then decanted into a 1000 mL measuring cylinder and the volume increased to 1000 mL. The sample was dispersed thoroughly for 30 seconds. Stokes' law shows that particles with a radius greater than 0.063 mm (sand) will take 47 seconds to settle from suspension in water at 20°C. Therefore specific gravity, representing the silt and clay proportions of the soil (g L^{-1}) was measured at this time (*j*). Stokes' law also show that particles with a radius of greater than 0.002 mm (silt) will take 5 hours to settle from suspension in water at 20°C. A further reading of specific gravity at this time represents the clay fraction of the soil (*n*). As liquid viscosity varies with temperature, temperature measurements taken in parallel with hydrometer readings were used to adjust specific gravity readings at both 47 seconds and 5 hours by ± 0.3 g L^{-1} for every degree above or below 20°C. By subtraction the relative proportions of sand (S), silt (Z) and clay (C) can be estimated, to two significant figures (Equations 2.4–2.6). The mass of sodium hexametaphosphate introduced was accounted for by addition of 0.3 g in Equation 2.3[4]

$$M = \left(50 - \left(\frac{\%\ \text{Loss on Ignition}}{100} \times 50.00\right)\right) + 0.3 \qquad (2.3)$$

$$\frac{j}{M} * 100 = \%C \qquad (2.4)$$

$$\frac{j-n}{M} * 100 = \%Z \qquad (2.5)$$

$$100 - (\%C + \%Z) = \%S \qquad (2.6)$$

[4] Mr sodium hexametaphosphate = 612 g mol
therefore 5 % solution contains 30.6 g L^{-1}
therefore 10 ml contains 0.306g

2. 5. Assessment of total organic and inorganic carbon, and total nitrogen

2. 5. 1. Theory

The experiments described in Section 2.4 determine the proportion of all organic matter that will oxidise at 405°C. This can only be used as an estimate of the soil carbon content as some organic compounds will not be fully oxidised at this temperature. There is also the potential for other elements to be oxidised during combustion. For a more accurate measure of soil carbon, quantitative oxidation and analysis using an elemental analyser was carried out. Due to the chemistry in this analysis data, the percentage nitrogen content of the soil can also be derived easily (FitzPatrick 1980).

2. 5. 2. Details of the method

For analysis of all forms of carbon and nitrogen approximately 75 g sample of air dried soil was milled to pass through a 500 μm sieve and then oven dried at 105 °C for 2 hours. Each sample is combusted explosively in a highly oxygenated atmosphere at 1800°C, oxidising the element carbon to CO_2, and nitrogen to NO_x and N_2. Helium is used as a carrier gas through the instrument and as a flush gas between each sample. A copper oxide catalyst is used to improve the efficiency of combustion. Volatile halogen compounds derived during oxidation are removed using silver wool prior to gasses entering the detection chamber. Quantitative detection was via a thermal conductivity detector, calibrated for the detection of N_2 and CO_2.

2.5.2(a) Total carbon and nitrogen

A sub-sample of approximately 100 mg was quantitatively reduced in an elemental analyser (vario EL, Elementar Americas Inc, USA).

2.5.2(b) Total organic carbon

Carbonates present in a 100 mg sub-sample were destroyed with HCl and dried for 4 hours at 90°C. Following this the sample was then quantitatively reduced in an elemental analyser (vario EL, Elementar Americas Inc, USA).

These readings allow the total N, total C and organic C contents of the sample to be calculated. From the latter two values the % total inorganic carbon can be inferred by subtraction. The ratio of total C to total N can also be calculated.

2. 6. An estimate of soil microbial biomass by fumigation extraction

2. 6. 1. Theory

The total size of the soil microbial community can be inferred by the detectable amount of soil organic carbon present in a sample after lysis of cell membranes on exposure to chloroform. This analysis is based on the British Standard 7755-4.4.2:1997 and the method described by Vance *et al.* (1987).

2. 6. 2. Details of the method

Samples were stored at 4°C for no longer than 7 days prior to homogenisation at field soil moisture content to pass through a 4 mm sieve. The soil moisture content was determined from a sub-sample.

Duplicate samples of 10.00 g soil were then weighed into separate vials designated fumigate (F) and control (C). Fumigation took place using a chloroform saturated atmosphere (HPLC grade) for 24 hours in a glass desiccator. Organic carbon was then extracted by shaking vials end-over-end with 40 mL 0.5 M K_2SO_4 for 45 minutes. The entire sample was then filtered through Whatman No. 42 filter papers. Blank treatments (B) were prepared by filtering 40 mL 0.5 M K_2SO_4 with no sample. Filtrates were then

stored at -17 °C prior to analysis using a SFA-2000 Segmented Flow Analyser (Burkard Scientific, Middlesex, UK). Defrosted samples were acidified and then sparged with CO_2-free air to remove inorganic carbon as CO_2. A two-fold dilution with acidified potassium persulphate (pH 2, adjusted with H_2PO_3) was then added and then irradiated with UV light, thus converting all organic carbon to CO_2. Evolved CO_2 was quantified by measurement of absorbance at 550 nm in buffered phenolphthalein and was recorded as dissolved organic carbon (DOC_F for fumigated sample and DOC_C for the control sample) for each duplicate sample. An estimate of microbial biomass C (MBC) (Equation 2.7) of the soil can then be determined using conversion factors (Wu *et al.* 1990; Beck *et al.* 1997):

$$MBC = \frac{DOC_F - DOC_C}{0.45} \qquad (2.7)$$

Final calculations were adjusted to compensate for soil moisture content within each sample used for analysis.

2. 7. Assessment of soil microbial community structure by analysis of phospholipid fatty acids

2. 7. 1. Theory

All living cells are surrounded by a lipid bi-layer: a membrane made from phospholipid fatty acids, cholesterols and proteins. Of these components phospholipid fatty acids (PLFA) are only found in living membranes and are hydrolised rapidly by cellular enzymes after cell death. The phenotype expressed by each fungal and bacterial cell found in the soil governs the composition of the PLFAs found in the cells membrane. This makes them an ideal marker from which to measure the phenotypic community structure of the soil microbial community. There are a wide range of PLFA molecules,

varying by structure and length of the lipid chains attached via a glycerol backbone to a polar head (Figure 2.2).

PLFA molecules are named by standard notation given as X:YωZ where X is the length of the carbon chain, Y is the number of double bonds and Z describes where the double bonds are in relation to the aliphatic methyl end of the molecule (ω). When double bonds are present, distinctions are made between cis and trans double bonds. Iso (*i*) and anteiso (*ai*) prefixes are used to describe branching methyl chains at the penultimate, or third carbon. The prefix *cyc* is used when the PLFA contains a cyclopropyl functional group. Hydroxyl fatty acids are designated *x*-OH with *x* signifying where the hydroxyl group can be found relative to the carboxylic end.

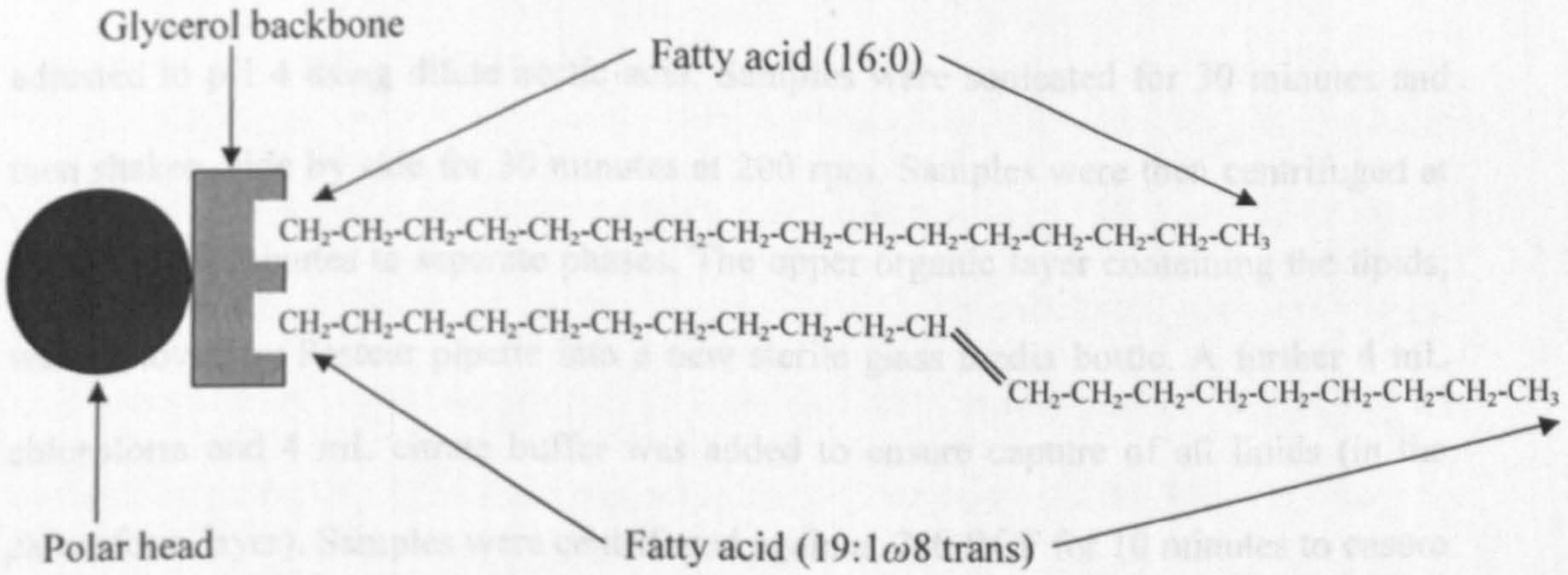

Figure 2.2: Schematic diagram of a generic phospholipid fatty acid.

2. 7. 2. Details of the method

The extraction procedure is based on a modified methods described by Frostegard *et al.* (1991), Bligh and Dyer (1959) and White *et al.* (1979). This method is descriptive of the soil microbial community structure and can not be used to measure community size (see Section 2.6)

2.7.2(a) Sample preparation

Samples were stored at 4°C for no longer than 7 days prior to preparation. Samples were homogenised at field soil moisture content to pass through a 4 mm sieve. Samples were then snap-frozen at -86°C and freeze dried using an Alpha 1-2 LD freeze dryer (Christ Freeze Driers, Osterode-an-Harz, Germany). This procedure preserves the lipid bi-layer of cells within the soil and so samples can be stored for prolonged periods of time at -17°C prior to laboratory analysis.

2.7.2(b) Laboratory analysis

Extraction

A 0.8:1:2 mixture of 0.15 M citrate buffer:chloroform:methanol (v:v:v) was mixed with 6 g of soil in a sterile glass media bottle, after Bligh and Dyer (1959). 0.15 M citrate buffer was made from 0.15 M citric acid dehydrate and 0.15 M trisodium citrate adjusted to pH 4 using dilute acetic acid. Samples were sonicated for 30 minutes and then shaken, side by side for 30 minutes at 200 rpm. Samples were then centrifuged at 700 *g* for 10 minutes to separate phases. The upper organic layer containing the lipids; was removed by Pasteur pipette into a new sterile glass media bottle. A further 4 mL chloroform and 4 mL citrate buffer was added to ensure capture of all lipids (in the chloroform layer). Samples were centrifuged again at 700 RCF for 10 minutes to ensure a clear interface between phases. The aqueous upper layer was removed and the organic layer was dried under a stream of nitrogen gas using a sample condenser. Lipid extracts were stored under nitrogen at -18°C until fractionation.

Fractionation

Fractionation of the extracted lipids in order to retain the polar lipids was carried out using commercially prepared solid phase extraction (SPE) columns (Sep-pak Vac, Waters Chromatography, Massachusetts, USA). Approximately 0.5 g anhydrous sodium

sulphate was added to the top of each cartridge to ensure it remained moisture free. Each cartridge was then washed with 2 mL chloroform and dried using a vacuum manifold. Cartridges were conditioned by adding a further 2 mL chloroform.

Lipid extracts were defrosted at room temperature with 1 mL of chloroform to re-suspend the extract prior to loading into the SPE cartridge. The neutral lipid fraction was eluted using 5 mL chloroform followed by the glycol lipid fraction using 12 mL acetone. Both of these fractions were discarded. The polar lipid fraction (including phosopholipids) were eluted into a sterile glass media bottle using 8 mL methanol. Samples were evaporated to dryness under a stream of nitrogen at 37 °C and then stored under nitrogen at -18 °C until mild alkaline methonolysis was carried out.

Gas chromatography preparation

Mild alkaline methanolysis cleaves the fatty acid from the phospholipid glycerol back bone (Figure 2.2) and replaces them with a methyl group which can easily be analysed using gas chromatography (GC) (Dowling *et al.* 1986). Phospholipids were defrosted at room temperature and re-suspended in 1 mL 1:1 toluene:methanol. 1 mL of 0.2 M methanolic potassium hydroxide was added to hydrolyse all lipids. The reaction was incubated at 37°C for 30 minutes then quenched using 0.25 mL 1 M acetic acid. A 5 mL aliquot of 4:1 hexane:chloroform (v:v) was added followed by 3 mL deionised water. Samples were then sonicated in a water bath for 30 minutes and centrifuged at 700 *g* for 10 minutes to separate phases. The lower aqueous phase was discarded. 3 mL of 0.3 M sodium hydroxide was added to wash out any underrivatised fatty acids. The non polar layer was filtered into a sterile glass media bottle through approximately 2 g sodium sulphate and evaporated to dryness under a stream of nitrogen gas at room temperature.

Fatty acid methyl esters (FAMEs) were stored under nitrogen at -18°C. FAMEs were defrosted at room temperature and reconstituted with 0.2 mL of hexane and transferred via Pasture pipette to GC vials and stored at -18 °C until analysis took place.

2. 7. 3. FAME determination and interpretation

All samples were analysed using an Agilent Technologies 6890N GC controlled by computer using Agilent G2070 ChemStation for GC systems (Agilent Technologies, California, USA). The GC was equipped with a slit/splitless auto-injector and a HP-5 capillary column; 30 m length, 0.32 mm internal diameter, 0.25 μm film (Agilent Technologies, California, USA). FAMEs were separated with a temperature program starting at 50 °C for 1 min, increasing at 25 °C min^{-1} to 160 °C, then 2 °C min^{-1} to 240 °C, followed by 25 °C min^{-1} until a final temperature of 310 °C was reached. The computer was programmed to inject 1 μL FAMEs sample (splitless) and used helium as a carrier gas (1 mL min^{-1}). Injection temperature was 310 °C. FAMEs were detected using a flame ionisation detector (FID) operating at 320 °C.

Standard mixtures of 32 know FAMEs (Sigma-Aldrich, Dorset, UK) were used to identify the retention time of the PLFAs of interest from each sample. Peak area from samples was recorded by integration at these pre-identified retention times on each sample's chromatogram. The relative concentration of each PLFA was expressed on a mol % basis. The following PLFA molecules (derived from FAMEs) were identified: 14:0; 14:1 isomer a; 14:1 isomer b; i15:0; ai15:0; 15:1; 15:0; 16:1 isomer; i16:0; 16:1ω7 c; 16:1ω7 t; 16:0; Me17:0 isomer; i17:0; ai17:0; 17:0 c; 17:1 isomer; 17:0; 17:0 isomer; 18:0 isomer; 18:2ω6 c; 18:1ω9 c; 18:1ω9 t; 18:1ω7 t; 18:1 isomer; 18:0; 19:2; 19:0 c; 19:0; 20:0. The biological significance of these markers can be found in Table 2.2.

Table 2.2: Indications of dominant microbial communities found in association with specific PLFA biomarkers (after Zelles *et al.* 1992; Zarnowski 2002; Keinanen *et al.* 2002; Pawlett 2002)

Associated dominant microbial community	Fatty acid group	PLFA inferred from FAME
Total bacterial abundance	Various	*i*15:0; *ai*15:0; 15:0; i16:0; 16:1ω7 t; *i*17:0; *ai*17:0; 17:0; cyc17:0; 18:0; 18:1ω7; *cyc*19:0
Gram negative bacteria	Mono unsaturated	16:1ω9 c; 16:1ω7 c; 16:1ω7 t; 18:1ω9 c; 18:1ω7 c; 18:1ω7 t
Gram positive bacteria, also associated with sulphur reducing gram negative bacteria	Saturated: terminally branched	*i*15:0; *ai*15:0; *i*16:0; *i*17:0
Sulphate reducing bacteria	Unsaturated: Branched and methyl branched	10Me16:0; *i*17:1ω7; *i*15:0
Gram negative bacteria, also associated with sulphur reducing gram positive bacteria	Cyclopropane	cyc17:0; cyc19:0
Actinomycetes	Methyl branched	10Me16:0; 10Me17:0; 10Me18:0
Type I methanotrophs	Mono unsaturated	16:0; 16:1ω8 c; 16:1ω5 c; 16:1ω5 t
Type II methanotrophs	Mono unsaturated	18:1ω8 c
Eucaryotes	Poly unsaturated	18:2ω6
Arbuscular mycorrhizal fungi	Mono unsaturated	16:1ω5
Green microalgae	Mono unsaturated	14:0; 16:0
Biofilms	Various	16:0 and 18:1ω7 c; 18:1ω9 c
Stress indicator		16:1ϖ7 t/16:1ϖ7 c

2. 8. Sampling sites

Five golf courses were selected for this study (and repeatedly sampled throughout this study). These courses are arranged in a roughly east-west transect within a 50 km radius of Cranfield University at Silsoe (Figure 2.3). The following golf courses were studied: John O'Gaunt Golf Club: John O'Gaunt Course (OSGB co-ordinates 522000, 247030); John O'Gaunt Golf Club: Carthagena Course (OSGB co-ordinates 521500, 248200); Woburn Golf and Country Club: Dukes Course (OSGB co-ordinates 491310, 233770);

Woburn Golf and Country Club: Marquess Course (OSGB co-ordinates 492690, 232940); Buckingham Golf Club (OSGB co-ordinates 467260, 233780).

These courses were selected due to the range of standards of play that take place on them, and the wide range of sand content, but narrow range of clay content of the surrounding soils (Figure 2.4). Paired courses at John O'Gaunt Golf Club and Woburn Golf and Country Club were used to reduce spatial variation within the overall data set.

Table 2.3: Types of surface construction found on the five different golf courses used through out this study.

Course	Types of play surface
Buckingham	Standard Tees; Fairways; USGA Greens; Temporary Greens
Carthagena	Standard Tees; Fairways; Standard Greens
Dukes	Standard Tees; Fairways; Standard Greens
John O'Gaunt	Standard Tees; Fairways; Standard Greens
Marquess	USGA topped tees; Fairways; USGA Greens

Significant differences between tees, fairways and greens at each golf course in all physicochemical parameters measured (see above) were highlighted using principal components analysis (PCA) and post-hoc one-way analysis of variance (ANOVA).

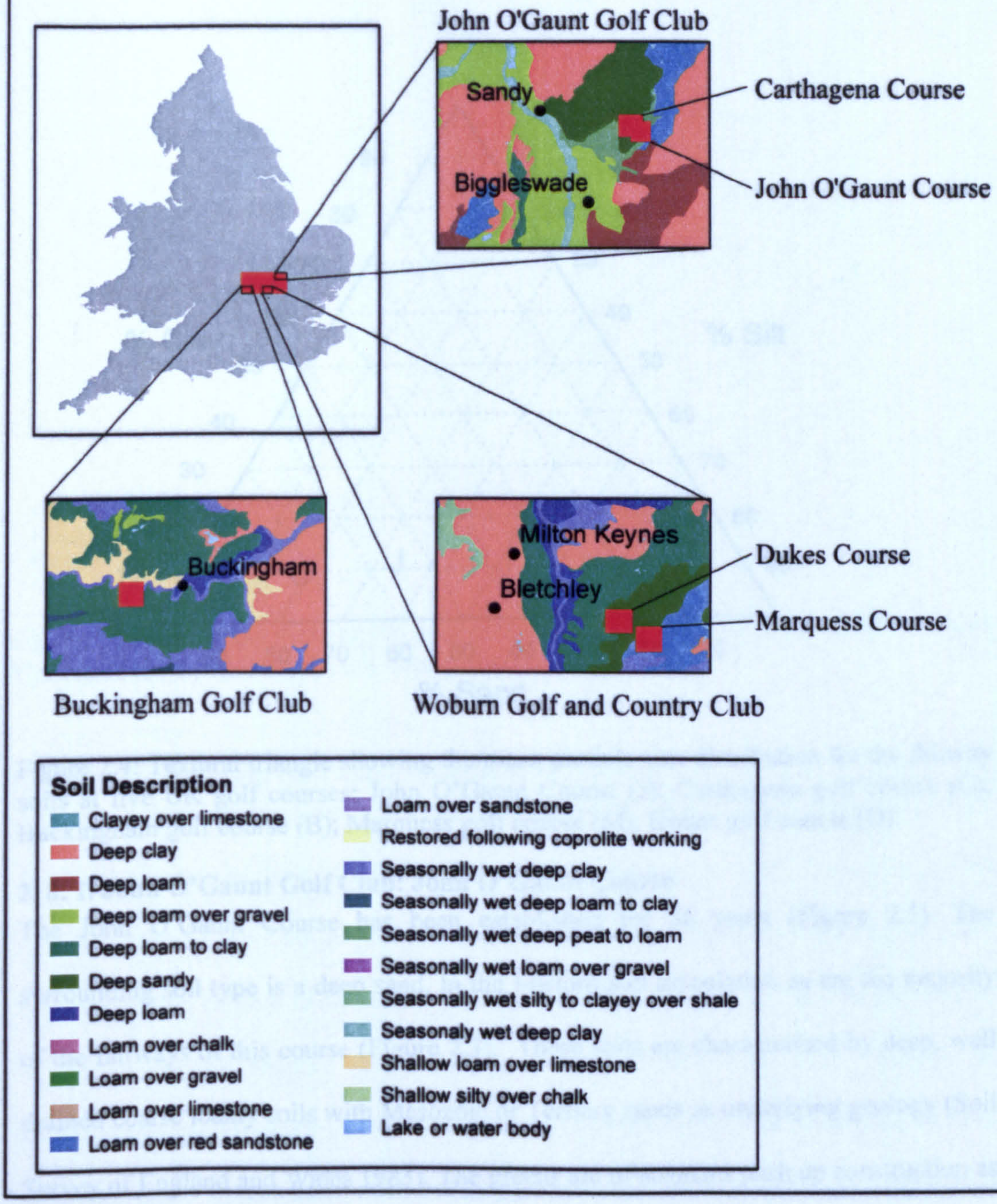

Figure 2.3: Map showing descriptive soil units and the five golf courses used through this study (Map derived from the National Soil Inventory of England and Wales, scale 1:250000)

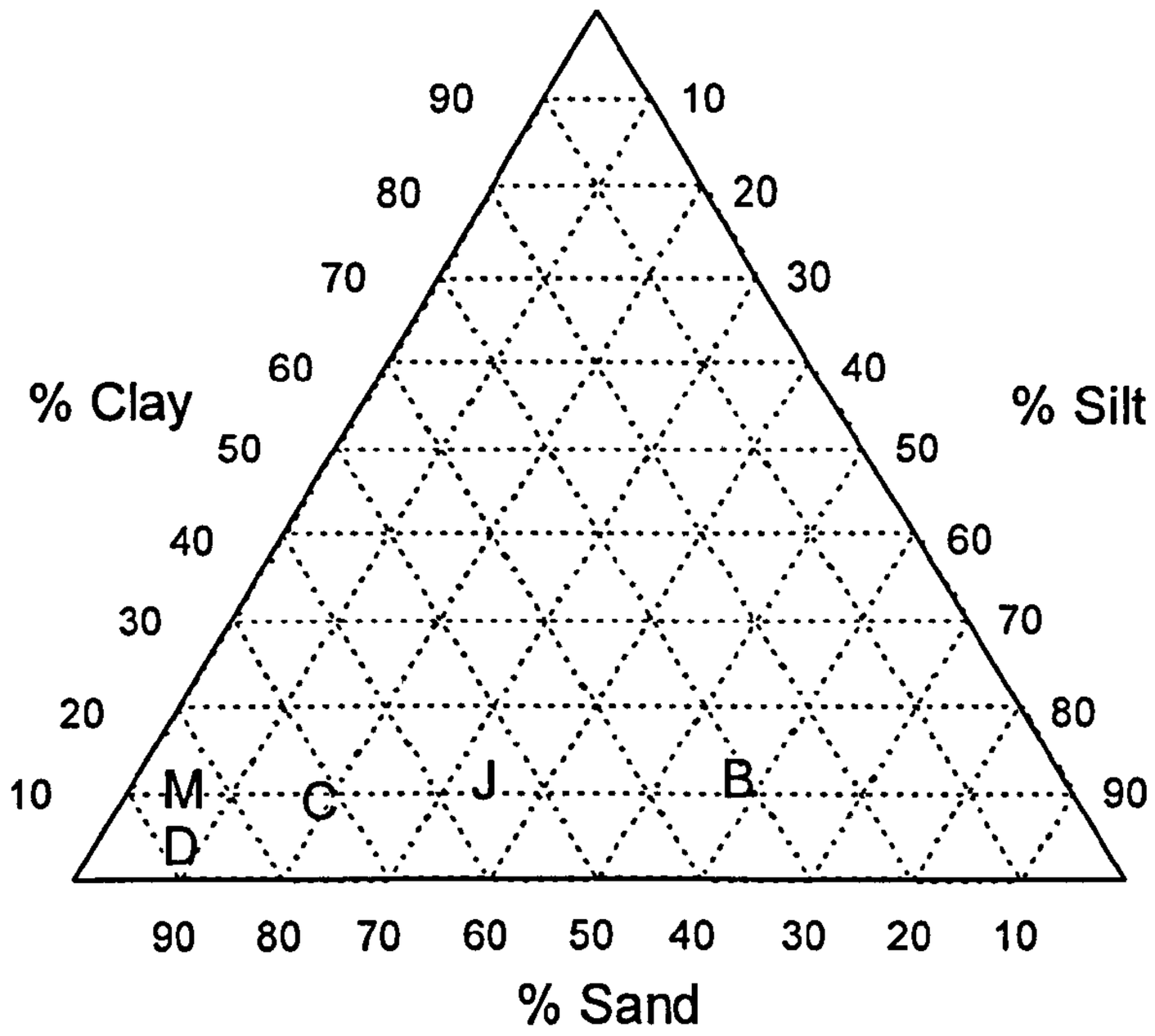

Figure 2.4: Textural triangle showing the mean particle size distribution for the fairway soils at five UK golf courses; John O'Gaunt Course (J); Carthagena golf course (C); Buckingham golf course (B); Marquess golf course (M); Dukes golf course (D).

2. 8. 1. John O'Gaunt Golf Club: John O'Gaunt Course

The John O'Gaunt Course has been established for 56 years (Figure 2.5). The surrounding soil type is a deep sand, in the Frilford soil association as are the majority of the fairways of this course (Figure 2.3). These soils are characterised by deep, well drained coarse loamy soils with Mesozoic or Tertiary sands as underlying geology (Soil Survey of England and Wales 1983). The greens are of standard push up construction as are the tees (Table 2.3). This course has twice been the venue for the English Golf Union Seniors Championships in 1993 and 2004 (John O'Gaunt Staff 2003).

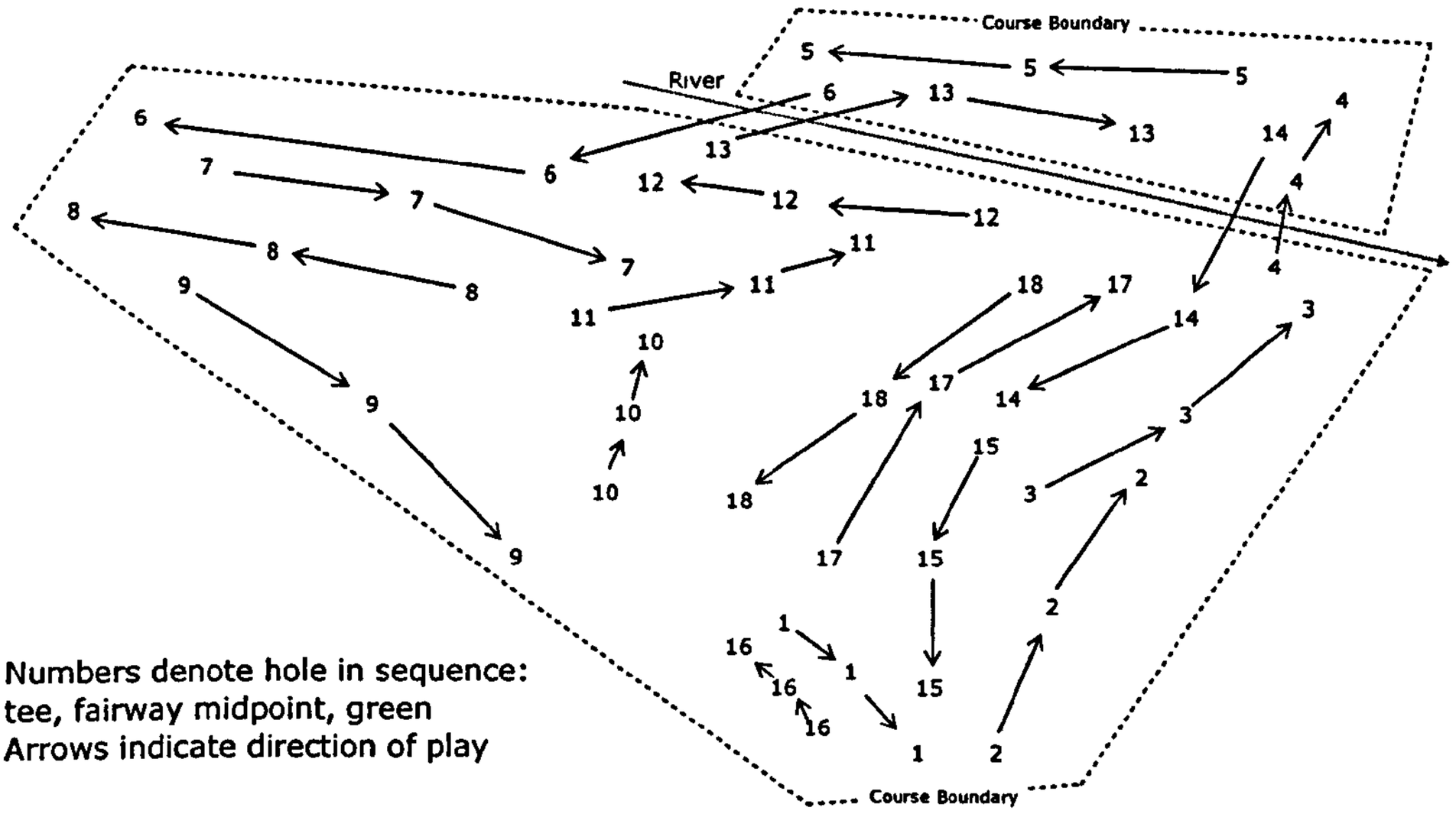

Figure 2.5: Pictograph showing the course layout and direction of play at John O'Gaunt Course, John O'Gaunt Golf Club. Not to scale.

2. 8. 2. John O'Gaunt Golf Club: Carthagena Course

The Carthagena course (Figure 3.3) at John O'Gaunt Golf Club was constructed on farmland adjacent to the original course, and was completed in 1995 (Figure 2.6). This course is also on the Frilford soil association, and so the soil of this golf course has a high proportion of sand (Soil Survey of England and Wales 1983). This course is close to boundaries of several other soil types (see Figure 2.3). The variation in physicochemical parameters is likely to be greater because of this. Both greens and tees are constructed as standard push up designs (Table 2.3). No national or international contests have been held on this course.

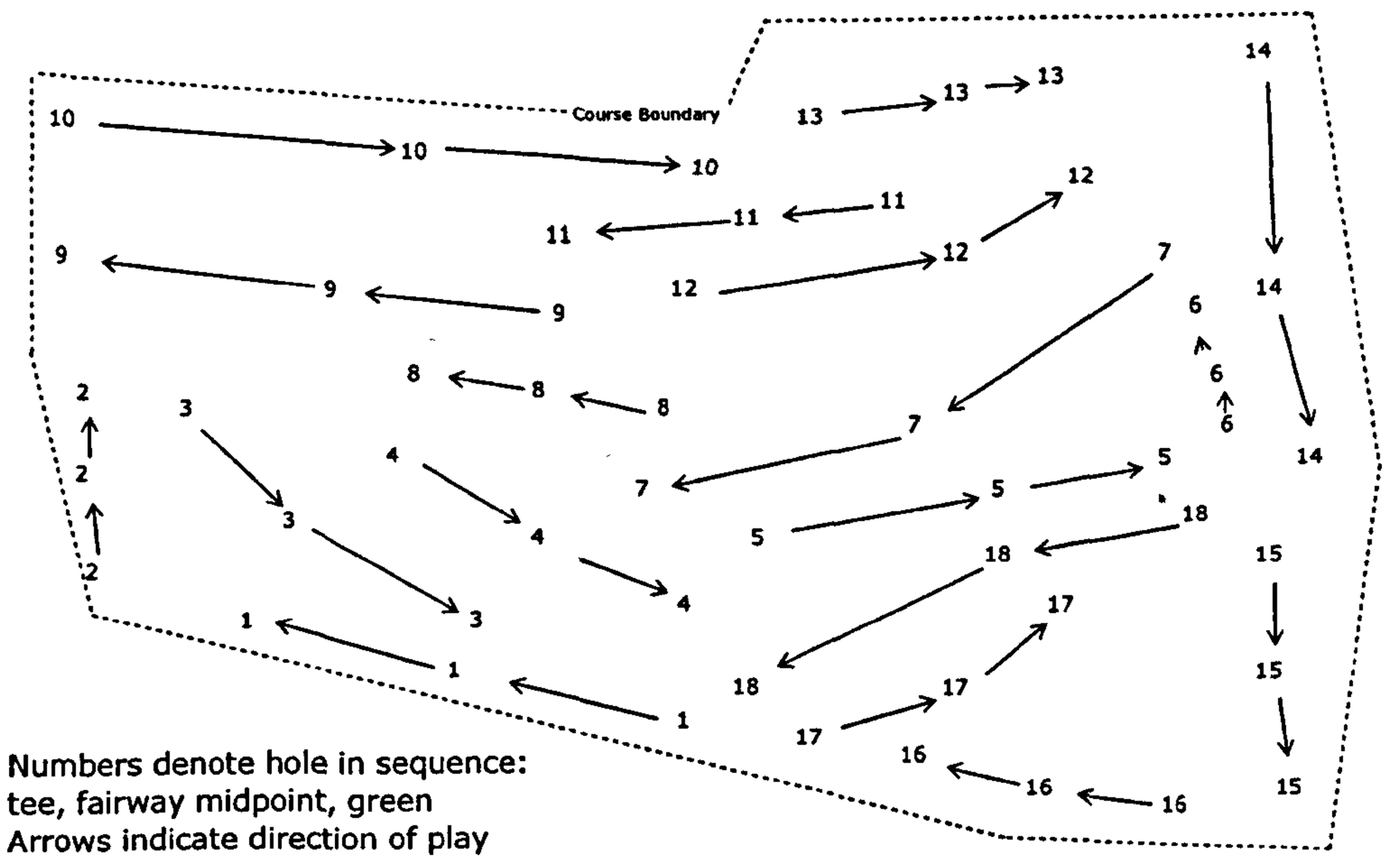

Figure 2.6: Pictograph showing the course layout and direction of play at Carthagena course, John O'Gaunt Golf Club. Not to scale.

2. 8. 3. Buckingham Golf Club Course

Buckingham golf club was established with a nine hole course in 1914, the present day course was expanded to eighteen holes in 1977 (Figure 2.7). The golf course is on the Fladbury soil association (Figure 2.3). This soil is characterized by stoneless clays from alluvial deposits (Soil Survey of England and Wales 1983). During 2004-2005 all 18 greens were upgraded from a push up construction to USGA specification greens. Tees are of standard construction (Table 2.3). No national or international competitions of note have been held at this course (Buckingham Golf Club Staff 2004).

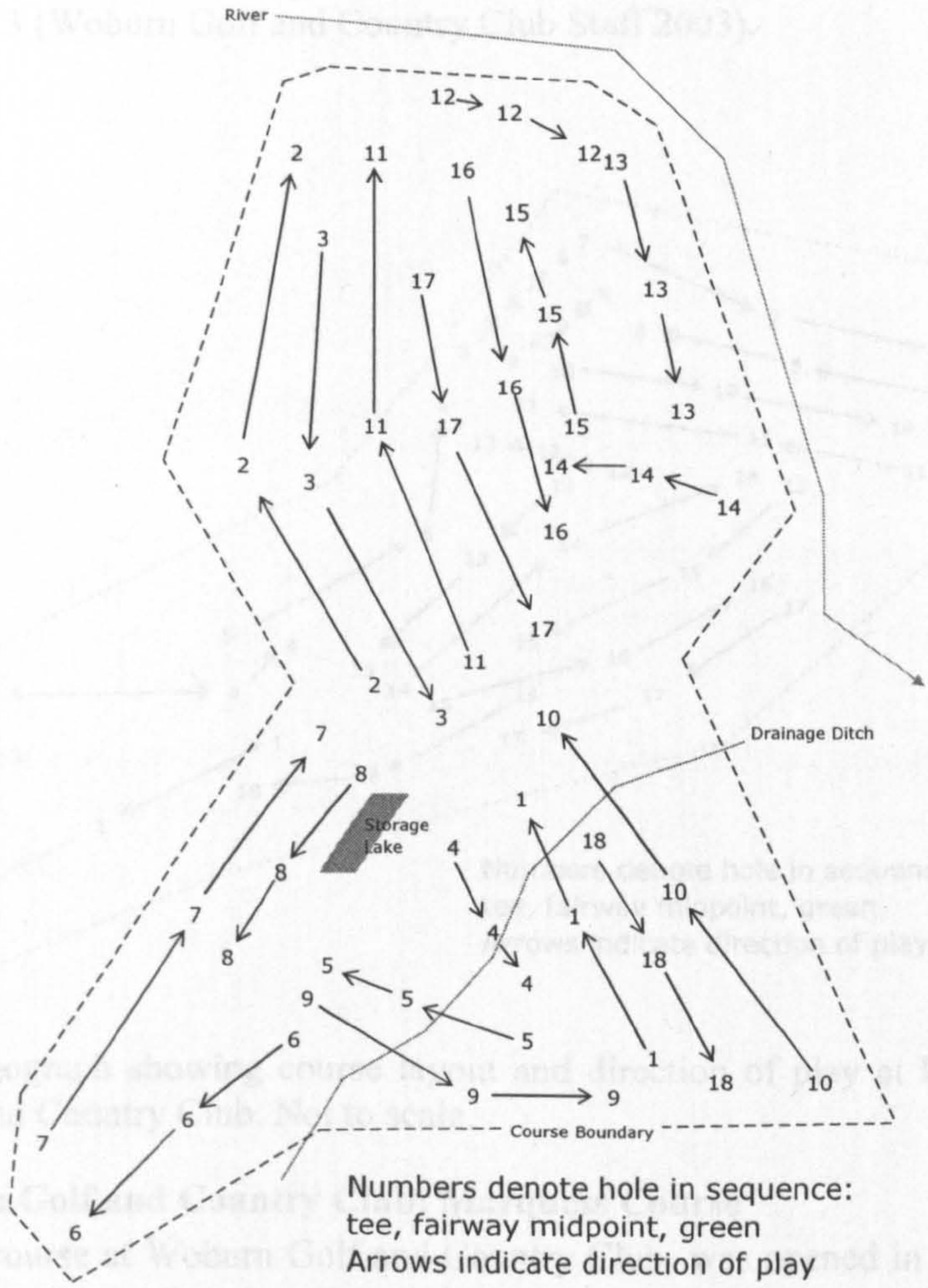

Figure 2.7: Pictograph showing the course layout and direction of play at Buckingham Golf Club. Not to scale.

2. 8. 4. Woburn Golf and Country Club: Dukes Course

The Dukes course at Woburn Golf and Country Club (Figure 2.8) was built 28 years ago to encourage world-class golf in the UK. This woodland soil is also of the Frilford association (Figure 2.3), a deep sandy soil over a Mesozoic or Tertiary sand (Soil Survey of England and Wales 1983). The course was used for the European Open on a number of occasions during the 1980's and 90's and 2005. The greens of this course are

standard push up construction but with perched water tables and tees are of standard design; Table 2.3 (Woburn Golf and Country Club Staff 2003).

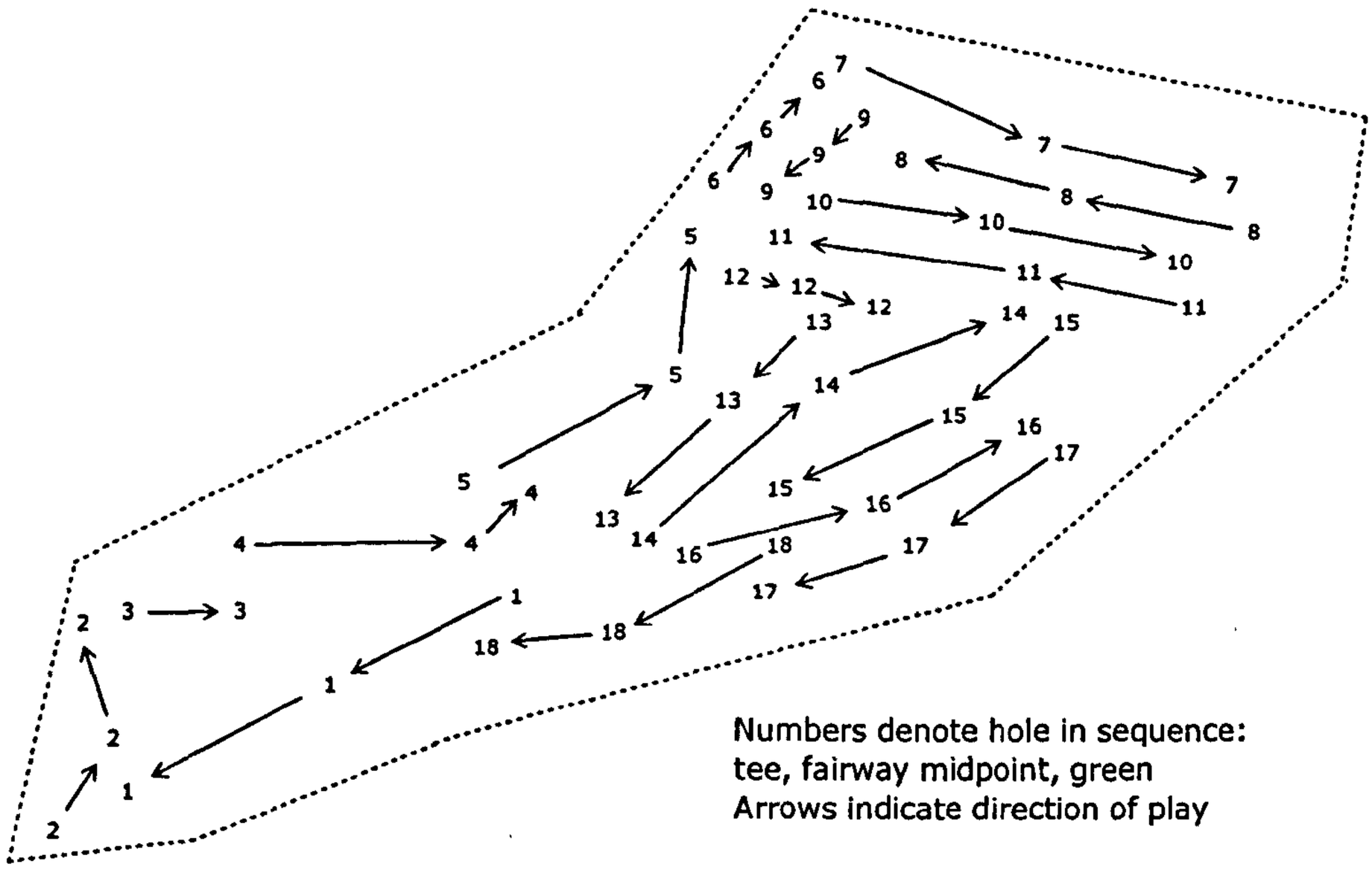

Figure 2.8: Pictograph showing course layout and direction of play at Dukes Course, Woburn Golf and Country Club. Not to scale.

2. 8. 5. Woburn Golf and Country Club: Marquess Course

The Marquess course at Woburn Golf and Country Club was opened in 2000 and was designed with modern championship contests in mind (Figure 2.9). This course is on the Bearsted soil association (Figure 2.3), a well drained sandy soil over Cretaceous sand and sandstone (Soil Survey of England and Wales 1983). Greens on this course are constructed to USGA specifications and tees are pushed up but also topped with USGA root zone material (70:30 sand:soil). It has been host to several national and international events since 2000 and is considered by many professional players to be the best inland course in Britain (Woburn Golf and Country Club Staff 2003).

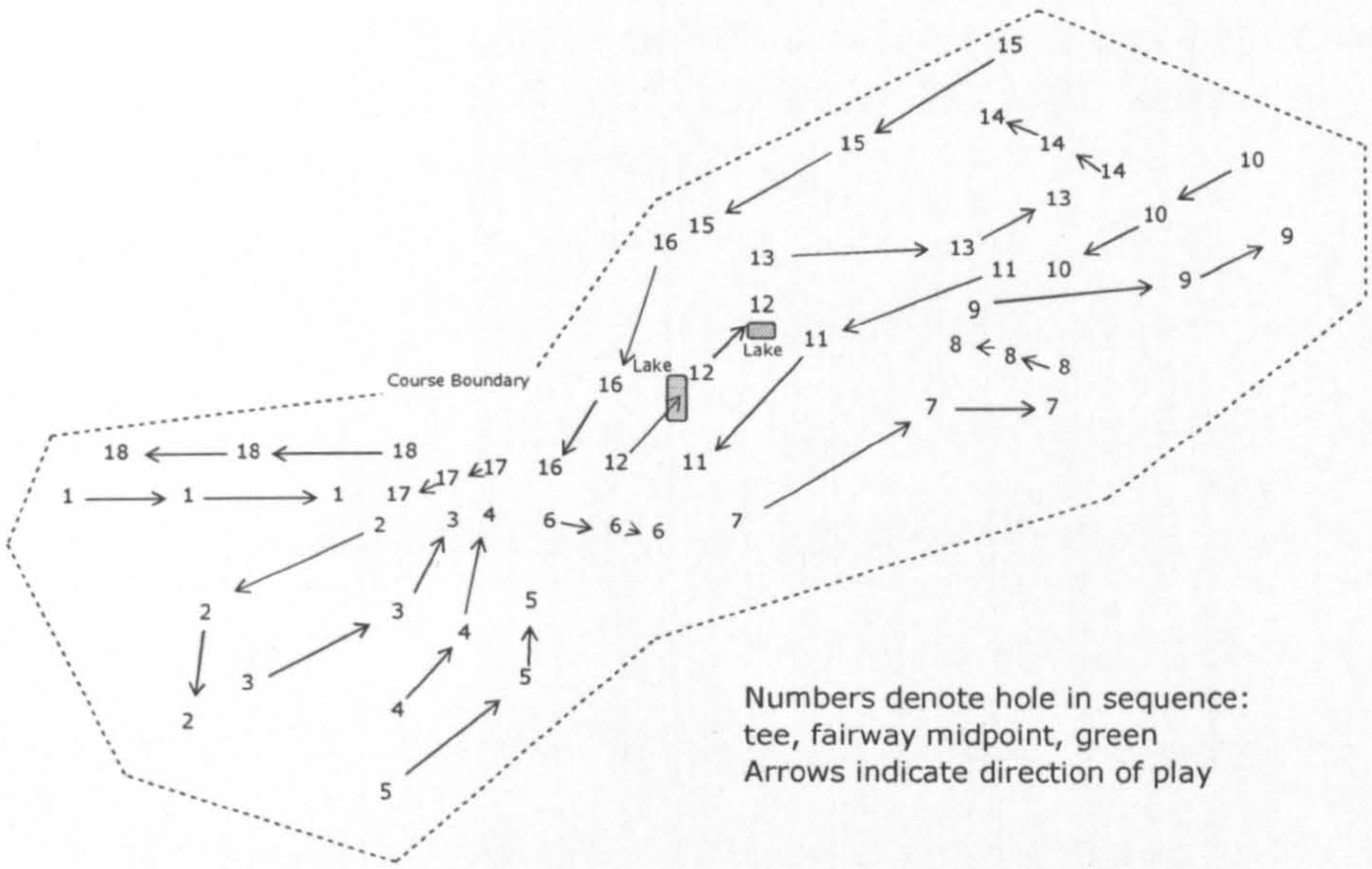

Figure 2.9: Pictograph showing course layout and direction of play at Marquess Course, Woburn Golf and Country Club. Not to scale.

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Chapter 3: The relationships between earthworm casting activity, season and play surfaces of golf courses at five golf courses in Bedfordshire and Buckinghamshire.

3. 1. Introduction

A range of chemical and physical control mechanisms have been proposed, and are used regularly in the control of earthworms on golf courses (Baldwin and Bennett 1990; Baker *et al.* 1998; Williamson 2004). However little quantitative research has taken place to characterise the activity of earthworms on different areas of a golf course at different times of year. This makes it impossible to quantifiably assess the success of any proposed control methods. The most comprehensive studies to date have been conducted by postal questionnaire (Baker *et al.* 1995; Baker and Binns 1998) or by analogy to other horticultural systems (Kirby and Baker 1995). Research carried out shortly after World War II made assessments of earthworm activity at 32 different golf courses. These experiments are limited by only being carried out in one year and one season (Jefferson 1956).

Assessing differences in temporal variation is an important component in any study of population or behavioural ecology (Vadermeer and Goldberg 2003). Earthworm reproductive cycles, and other biological processes and activities have been shown to vary with time of year and a range of other soil physicochemical properties (Boag *et al.* 1997; Baker *et al.* 1998; Butt and Nuutinen 1998). Long term experiments are therefore essential in characterising the dynamic change in earthworm activity at different times of the year. Physicochemical and soil moisture characteristics are important co-variables in determining the relationships of earthworm activity and environment.

All surveys of earthworm activity are complicated further due to the level of spatial variation associated with earthworm communities. Darwin (1883) first noted "...even on the same field worms are much more frequent in some places than in others, without any visible differences in the nature of the soil". This spatial variability makes it difficult to relate either indicators of earthworm activity or earthworm species to individual environmental parameters (Poier and Richter 1992). This means that direct measurement of earthworm activity in this highly managed ecosystem is required (Heemsbergen *et al.* 2004; Postma-Blaauw *et al.* 2006). Most experiments that have been carried out to determine levels of spatial variation in earthworm populations have been conducted at a single field scale and generally fail to provide causal mechanisms relating to distributions (Nuutinen *et al.* 1998). They also indicate that to minimise this variation large experimental designs are required to test any hypotheses relating to the activity of earthworms.

It is well documented that digging sample pits to survey earthworm populations is the most accurate method of population assessment (Edwards and Lofty 1977; Lee 1985; Gunn 1992; Lawerence and Bowers 2002) and the use of chemical expellance methods is the next most effective (Lawerence and Bowers 2002). However, both these methods have inherent biases (Gunn 1992; Chan and Munro 2001; Zaborski 2003; Bartlett *et al.* 2006). In a golf course environment both methods have major drawbacks: the digging of appropriate sized pits (0.027 m^3, i.e. 0.3 x 0.3 x 0.3 m) is too disruptive and the number of sample pits that would be required also makes this technique untenable. No guarantees that turfgrass will not be damaged can be given for expellance methods and so are inappropriate for measuring earthworm populations in all areas of the golf course

where the aesthetic appearance of the turf is important. Anecic earthworms form casts on the surface, making such features an effective surrogate measure of the levels of activity of this ecological group of earthworms. A survey assessing the frequency of casts and smears (partially broken down casts) was used as a rapid method of determining earthworm activity (Rossi and Nuutinen 2004). Using this method, all eighteen tees, fairways and greens at a golf course can be surveyed within one day. Both casts and smears need to be assessed because this is a direct measure of the scale of the problem from the green-keeper's perspective. Soil mechanical investigations suggest that earthworm casts formed up to 10 days prior to the sampling point are likely to be stable and identifiable, thus make a good proxy for measurement of earthworm activity (Le Bayon and Binet 2001).

To test the following hypotheses a large long term experiment was required:

Hypothesis 3.1. The activity of earthworms on different surfaces of golf courses varies with the surface construction due to the differing physicochemical parameters related to each surface.

Hypothesis 3.2. Earthworm activity increases during the spring and autumn months on all areas of golf courses relating to the life-cycle of earthworms.

Hypothesis 3.3. Annual variations in earthworm activity are related to the local environmental meteorological changes as earthworms respond to the changing gradients in these environmental parameters.

3. 2. Materials and Methods

3. 2. 1. Field Methodology

Surveys were carried out every other month (bimonthly) for 24 months between June 2004 and May 2006 at each of the five golf courses described in Chapter 2.8 (i.e. n = 12 sampling events per course). An assessment of earthworm cast and smear densities was made on each surface sampled and recorded as $\Sigma_{cast+smears}$. Sampling every two months ensures that most of the casts and smears have broken down since the last sample time (Decaens 2000) and still maintains an extremely high resolution assessment of earthworm casting behavioural activities in relation to golf courses. The size of the areas surveyed and the survey design means the probability that one cast would be observed twice in the study was negligible.

At each sampling event every hole was sampled on every surface used for play; n = 54 per course[5]. These surfaces can be generically divided into tees, fairways and greens. However, site-specific surface construction was taken into account (Table 2.3). From these surfaces six treatments (in a statistical sense) were denoted:

- Standard tee (designated T)
- USGA topped tee (designated UT)
- Fairway (designated F)
- Standard green (designated G)
- USGA green (designated UG)
- Temporary green (designated TG)

[5] n=53 at the Dukes course, Woburn golf and country club due to course design, see Figure 2.11.

All surveys were made using a quadrat (0.5 x 0.5 m; 0.25 m^2) in a stratified random design. Some work has been carried out to determine the optimal size quadrat for the assessment of earthworm casts (Rossi and Nuutinen 2004) and concluded that no major differences in assessment result in using a quadrat of this size when compared to a larger (1.0 m^2) quadrat. Stratification within the survey was delimited by tees, fairways and greens. Only the men's competition tees were used on each hole of each golf course. Nine replicates are made on the tees and greens with a 'W of best fit' across the tee or green area. The fairway was sub-stratified, by dividing it into ten equally sized strata and three randomly placed quadrats being taken from within each area (Figure 3.1; n ≈ 540 per course per sampling event). Any potential increase in variance as a result of using a 0.25 m^2 quadrat, a possibility highlighted by Rossi and Nuutinen (2004), was deemed acceptable due to the increase in sampling effectiveness when taking such a large number of replicates at each sampling event. Soil moisture was also recorded at every third quadrat using a HH2 theta probe (Delta T devices, Cambridge, UK).

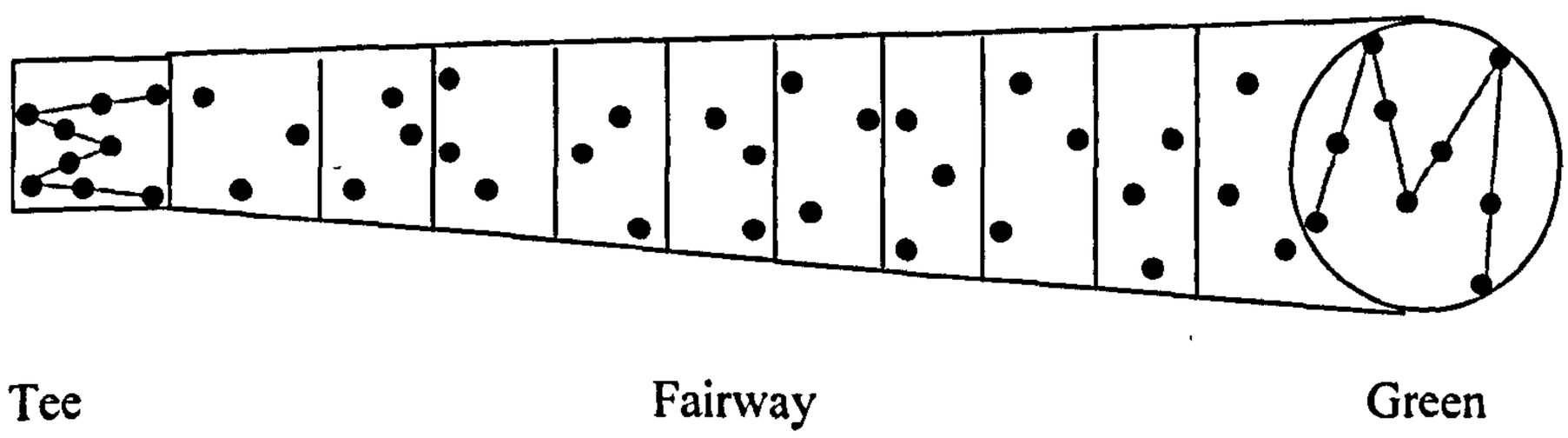


Figure 3.1: Stratifications and sub stratifications for cast surveys

The effect of the management decisions of the green-keeper on earthworm activity is important in this length of experiment. All green-keepers were requested to make no change in their behaviour relating to earthworm controls for the duration of the study.

No data was released to green-keepers during the survey period to avoid biasing sampling.

3. 2. 2. Physicochemical factors

Soil samples were taken from each surface surveyed during March 2005 using a soil auger (25 mm diameter, 250 mm deep). Five soil cores were taken per surface and pooled for analysis (n = 287). Measurements were made following methodologies set out in Chapter 2 to be used as co-variates in spatial analysis of earthworm activity distributions.

- Total cation exchange capacity (CEC)
- pH
- % Nitrogen (N)
- % Total carbon (C)
- % Total organic carbon (TOC)
- % Sand
- % Clay
- % Silt
- C:N, derived from % total C and % nitrogen

3. 2. 3. Environmental (weather) factors

In addition to the soil moisture measured using a theta probe on each surface while each quadrat survey was carried out, climatic data was taken from the automatic weather station based at Cranfield University at Silsoe. All golf courses are within a 50 km radius of this point (see Figure 2.3). The following parameters are used as co-variables in analysis.

- Maximum air temperature (T_{max}, °C)

- Minimum air temperature (T_{min}, °C)
- % Relative humidity (RH)
- Evapotranspiration (ET, mm d^{-1})
- Rainfall (mm d^{-1})

Each of these variables used in the analysis was taken as a mean of the readings seven days prior to sampling date. While the ideal would be to have meteorological station on each golf course for resource and logistical reasons this was not possible.

3. 2. 4. Statistics and models

Generalized linear models (GZM) were used to estimate mean earthworm activity (mean $\Sigma_{cast+smears}$ m^{-2}) on different surfaces and at different times of year. Post-hoc differences were distinguished using 95% confidence intervals. Physicochemical data was analysed using principal components analysis (PCA) using correlations with subsequent one way analysis of variance (ANOVA) on each principal component to detect any relevant structures within the data. Predictorial relationships between average earthworm activity (from GZM results) and both physicochemical and environmental variables were derived using linear forward stepwise multiple regression. All data modelling was carried out in Statistica 7.1 (Statsoft Inc. 2005).

3. 3. Results

3. 3. 1. Distribution

The observation of earthworm casts on a golf course is an infrequent event; therefore the data recorded over 24 months of the survey is severely non-normally distributed. A Poisson distribution was confirmed using VassarStat; Figure 3.2 (Lowry 2005). Observed vs. expected frequencies proportion of linear covariance, describing the quality of the fit of the data, measured as $r^2 = 0.999$.

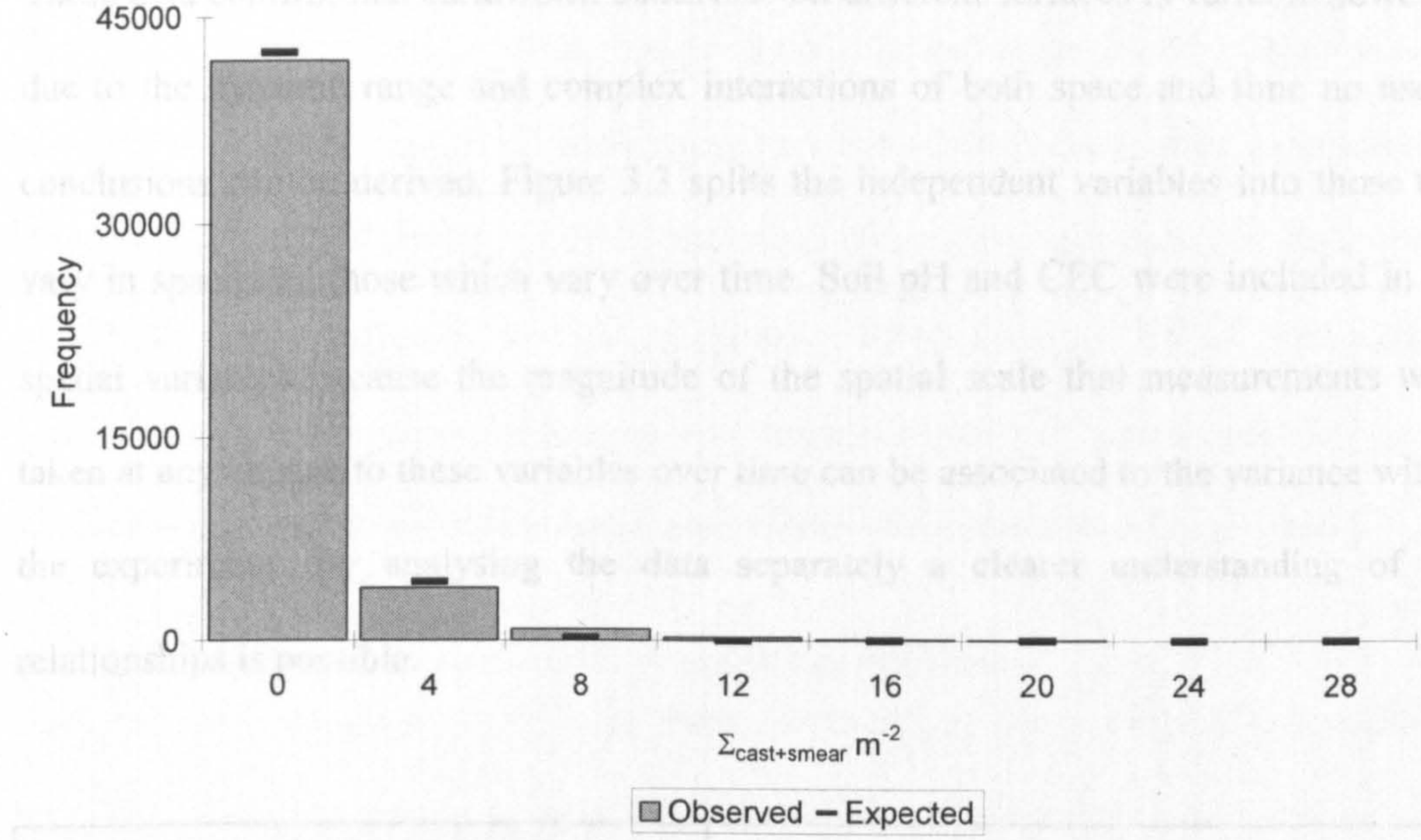


Figure 3.2: Histogram of distribution of $\Sigma_{cast+smears}$ m^{-2} data recorded throughout the two year study

3. 3. 2. Space x time x location interaction

On each individual hole on a golf course there is only one area designated tee, one fairway and one green. Therefore relationships at the broadest level of surface (n=3) per course (n=5) at different times of year (n=6) can be investigated, irrespective of surface constructions. Highly significant relationships and interactions are evident with all independent variables ($p < 0.01$ for all variables and interactions).

Table 3.1: Results of a space x time x location GZM analysis.

Independent variable	Degrees of freedom	Wald χ^2	p
Intercept	1	5298.1	< 0.01
Time	5	2019.8	< 0.01
Course	4	1311.5	< 0.01
Surface	2	626.1	< 0.01
Surface x Course	8	2706.3	< 0.01
Time x Course	20	1033.1	< 0.01
Time x Surface	10	239.6	< 0.01
Time x Course x Surface	40	2111.3	< 0.01

These data confirm that earthworm behaviour on different surfaces is variable however, due to the dynamic range and complex interactions of both space and time no useful conclusions can be derived. Figure 3.3 splits the independent variables into those that vary in space and those which vary over time. Soil pH and CEC were included in the spatial variables because the magnitude of the spatial scale that measurements were taken at any change to these variables over time can be associated to the variance within the experiment. By analysing the data separately a clearer understanding of the relationships is possible.

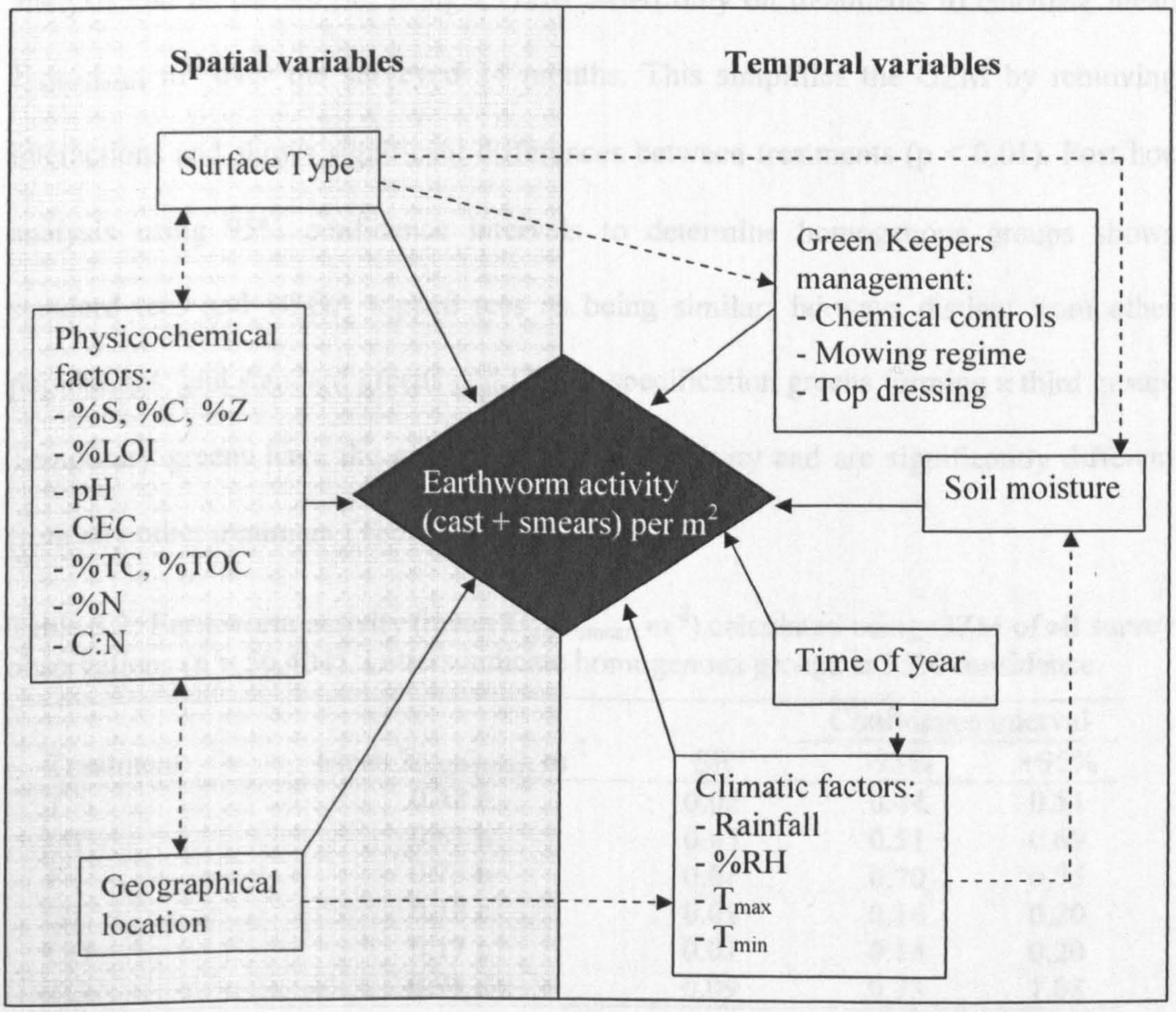


Figure 3.3: Variables and their interactions which have potential to affect the earthworm activity on a golf course

3.3.2(a) Physicochemical similarities between surfaces

PCA of all physicochemical parameters on the 287 surfaces used for the quadrat surveys shows that there are significant differences between surfaces designated as treatments. These differences are independent of the surfaces geographical location. Figure 3.4a shows discrete populations in PC1 independent of location for USGA specification greens and USGA topped tees; temporary greens; standard tees, greens and fairways ($p < 0.01$). Each of these groupings in the data is further separated in PC2 ($p < 0.01$).

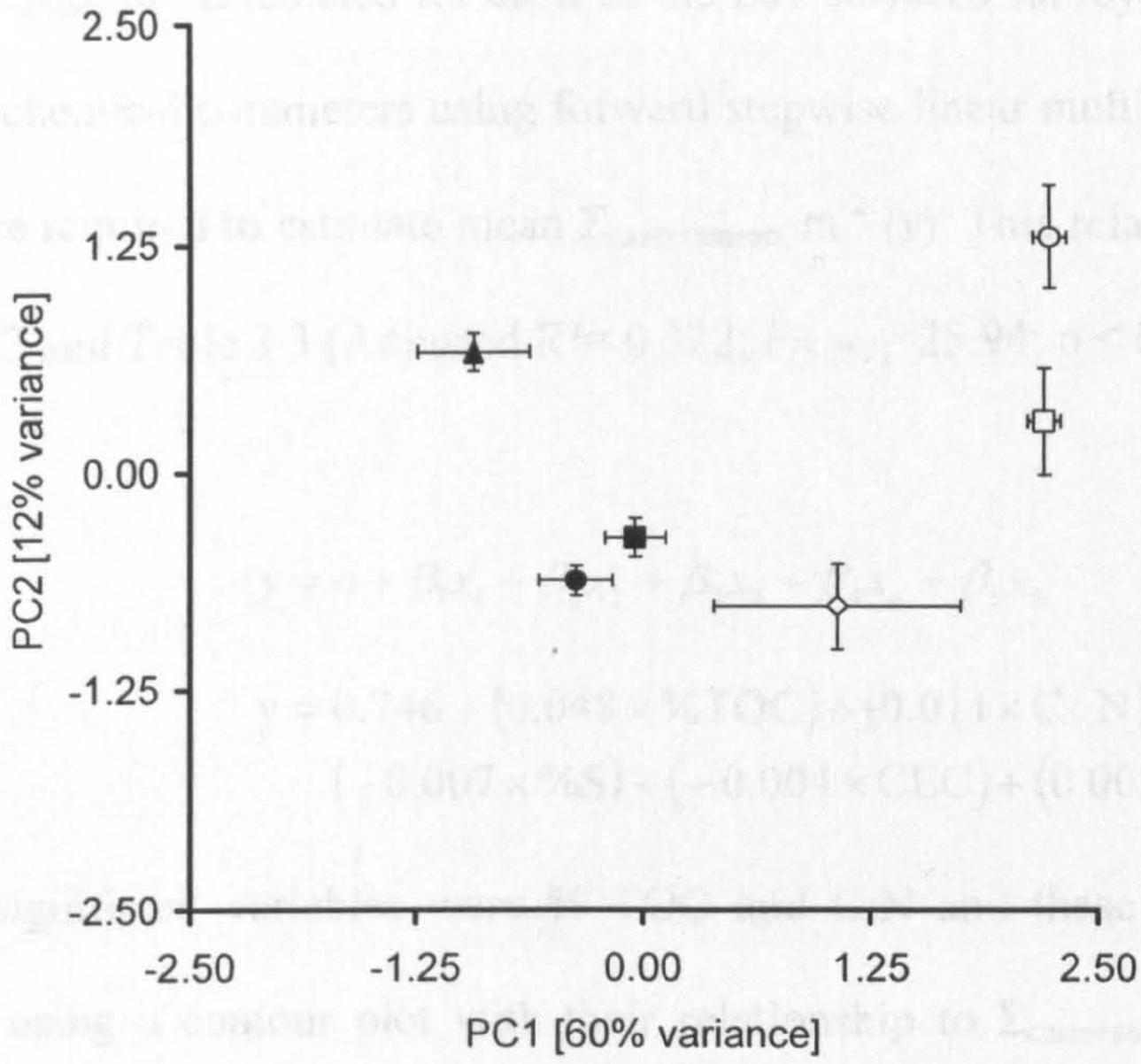
3. 3. 3. Predicting earthworm activity on different surfaces

Each surface is significantly different, independent of golf course (Figure 3.4), therefore analysis can be carried out using a GZM based only on treatments to calculate mean $\Sigma_{casts+smears}$ m^{-2} over the surveyed 24 months. This simplifies the GZM by removing interactions and shows significant differences between treatments ($p < 0.01$). Post hoc analysis using 95% confidence intervals to determine homogenous groups shows standard tees and USGA topped tees as being similar; fairways distinct from other populations; and standard greens and USGA specification greens forming a third group. Temporary greens have the greatest earthworm activity and are significantly different from any other treatment (Table 3.2).

Table 3.2: Earthworm activity (mean $\Sigma_{casts+smears}$ m^{-2}) calculated using GZM of all survey observations (n = 50,404). Letters indicate homogenous groups at 95% confidence.

			Confidence interval	
Treatment	Mean $\Sigma_{casts+smears}$ m^{-2}	SE	-95%	+95%
T	0.48 a	0.02	0.44	0.51
UT	0.61 a	0.05	0.51	0.69
F	0.73 b	0.01	0.70	0.75
G	0.18 c	0.01	0.16	0.20
UG	0.17 c	0.01	0.14	0.20
TG	0.91 d	0.09	0.73	1.08

a)



b)

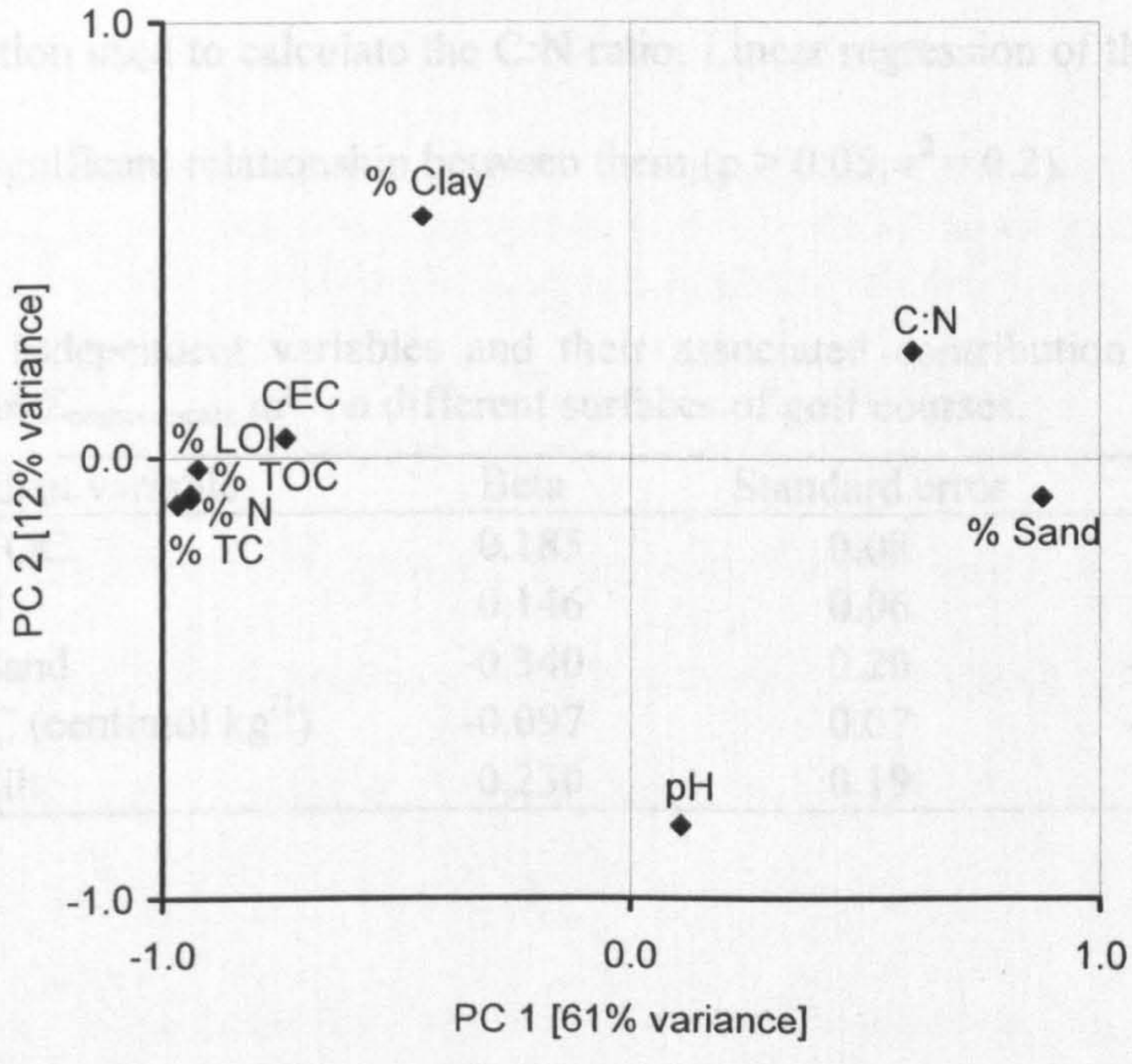


Figure 3.4: Principal Component (PC) analysis of physicochemical data from five golf courses. a) Projection of mean co-ordinates of significant PCs with respect to standard tees (●), USGA tees (○), Fairways (▲), standard greens (■), USGA greens (□) and temporary greens (◊) found on golf courses. All whiskers show standard error of the mean. b) Loading values from PCA of physicochemcial data from five golf courses attributed to differences between surfaces projected in Figure 3.4a.

Mean $\Sigma_{casts+smears}$ m^{-2} calculated for each of the 287 surfaces surveyed can be related to the physicochemical parameters using forward stepwise linear multiple regression. Five variables are required to estimate mean $\Sigma_{casts+smears}$ m^{-2} (y). This relationship is given by Equation 3.2 and Table 3.3 (Adjusted R^2= 0.322; $F_{(5,257)}$=25.94; $p < 0.01$):

$$y = a + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 \quad (3.1)$$

$$\begin{aligned} y = {} & 0.746 + (0.048 \times \%TOC) + (0.011 \times C:N) + \\ & (-0.007 \times \%S) + (-0.004 \times CEC) + (0.005 \times \%Z) \end{aligned} \quad (3.2)$$

The most significant variables were % TOC and C:N and these can be graphically interpreted using a contour plot with their relationship to $\Sigma_{casts+smears}$ m^{-2}, Figure 3.5. These variables have a degree of inter-dependency: % total organic carbon is part of the carbon fraction used to calculate the C:N ratio. Linear regression of these two variables shows no significant relationship between them ($p > 0.05$; $r^2 = 0.2$).

Table 3.3: Independent variables and their associated contribution to the model in predict mean $\Sigma_{casts+smears}$ m^{-2} on different surfaces of golf courses.

Independent variable		Beta	Standard error	$t_{(257)}$	p
β_1	% TOC	0.185	0.08	2.451	0.01
β_2	C:N	0.146	0.06	2.412	0.02
β_3	% Sand	-0.340	0.20	-1.701	0.09
β_4	CEC (centimol kg^{-1})	-0.097	0.07	-1.351	0.18
β_5	% Silt	0.230	0.19	1.183	0.24

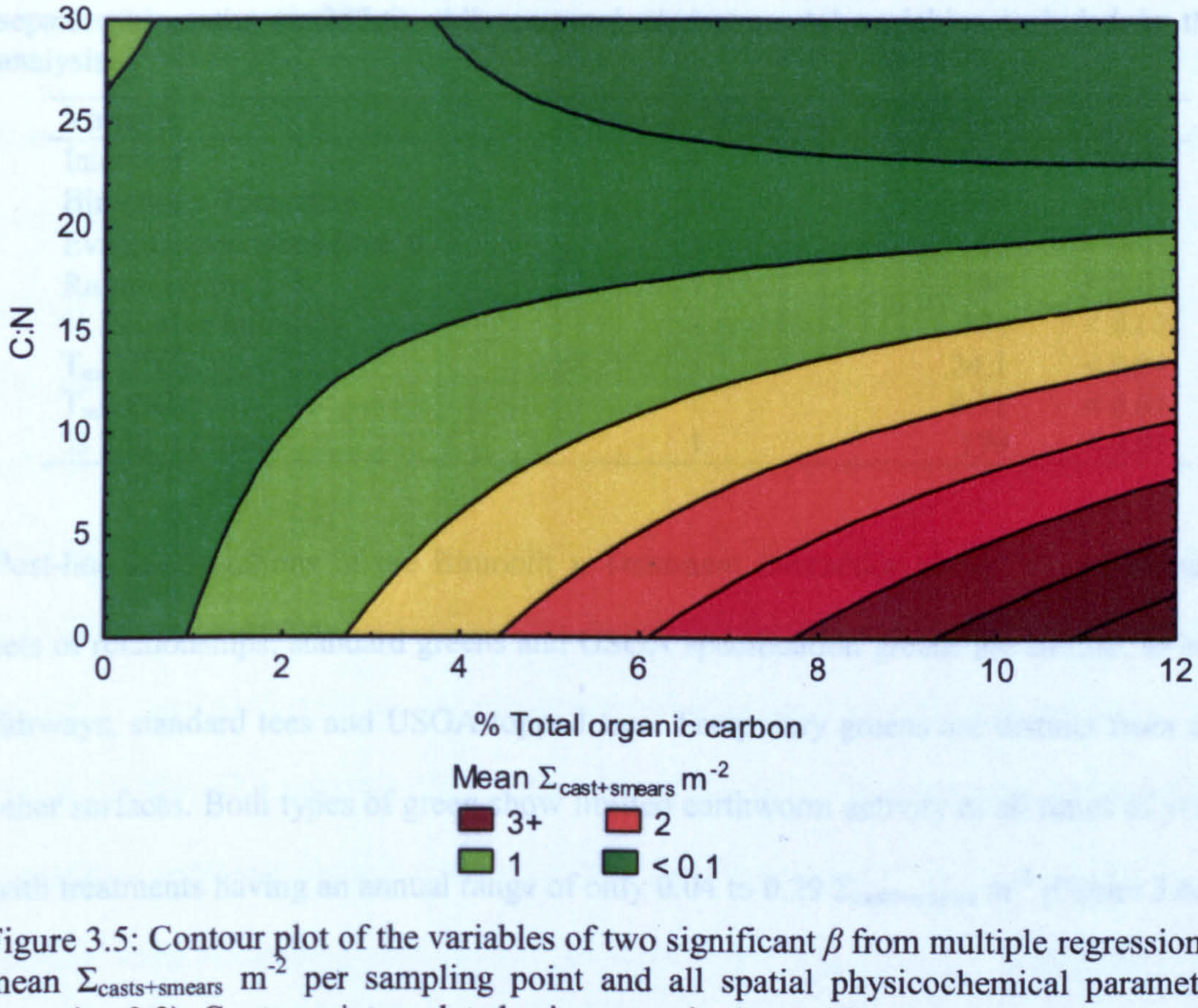


Figure 3.5: Contour plot of the variables of two significant β from multiple regression of mean $\Sigma_{casts+smears}$ m^{-2} per sampling point and all spatial physicochemical parameters (equation 3.2). Contours interpolated using a quadratic model.

3. 3. 4. Predicting earthworm activity at different times of year

The strength of the relationships between earthworm activity and different surfaces means that any interpretation of variation in activity at different times of year must include the surface as a treatment. The inclusion of this variable also means that the course variable can be eliminated due to the homogeneity of surfaces at disparate sites (Figure 3.4). Environmental parameters, averaged for seven days preceding sampling are included as co-variables (see Section 3.2.3) and even when this variation is accounted for ($p < 0.01$ in all cases; Table 3.4) significant differences still remain between treatments at different times of year.

Table 3.4: Significant interactions from GZM of $\Sigma_{cast+smears}$ m^{-2} on different surfaces in separate bimonths (n=287,6). All temporal environmental variables included in the analysis.

Variable	Degrees of Freedom	Wald χ^2	p
Intercept	1	504	< 0.01
Bimonth x Treatment	25	3240	< 0.01
Evapotranspiration (mm d^{-1})	1	1160	< 0.01
Rainfall (mm d^{-1})	1	1200	< 0.01
% Relative humidity	1	774	< 0.01
T_{max} (°C)	1	74.1	< 0.01
T_{min} (°C)	1	9.71	< 0.01
% Soil moisture	1	179	< 0.01

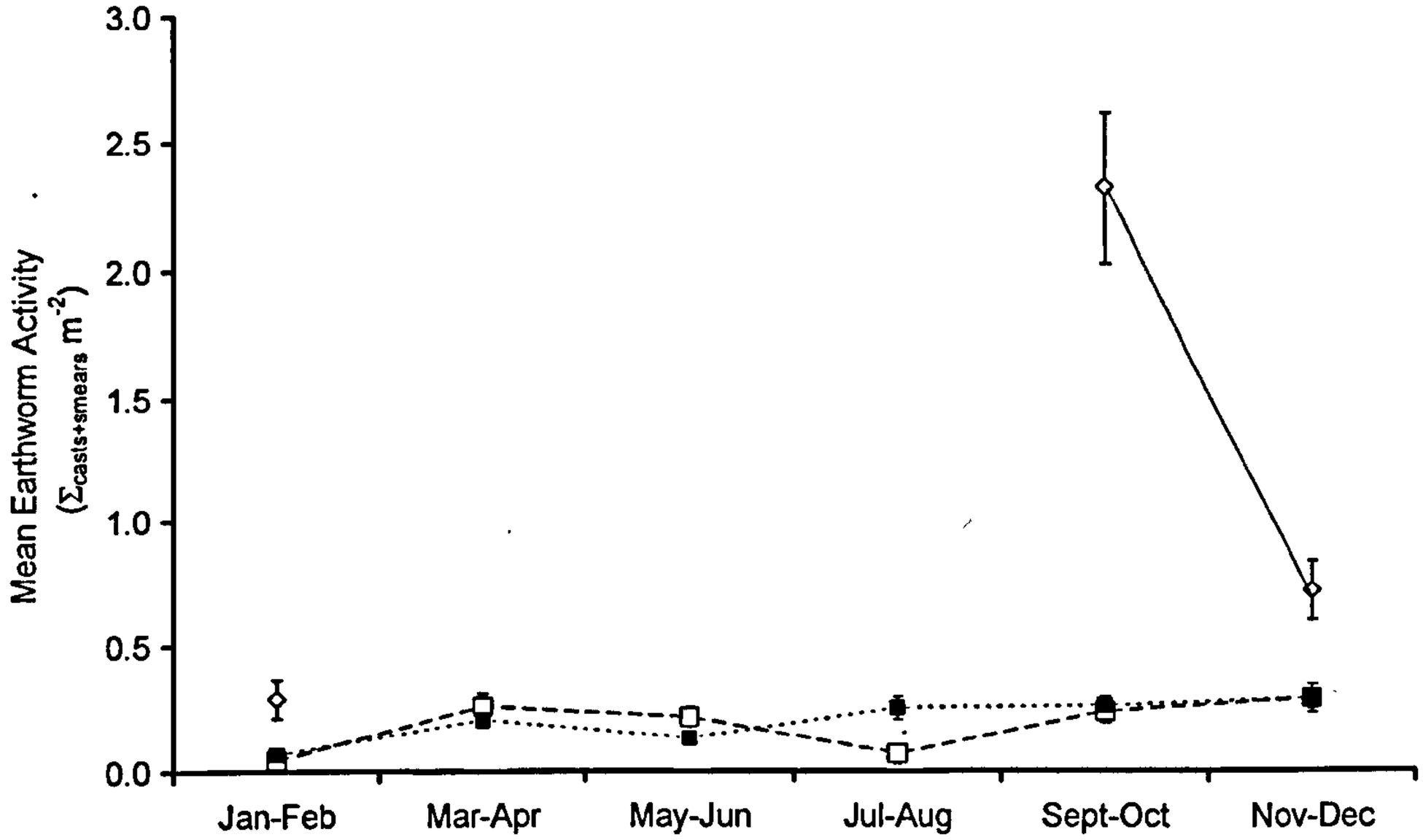
Post-hoc investigations of the Bimonth x Treatment interaction shows three different sets of relationships: standard greens and USGA specification greens are similar, as are fairways, standard tees and USGA topped tees. Temporary greens are distinct from all other surfaces. Both types of green show limited earthworm activity at all times of year with treatments having an annual range of only 0.04 to 0.29 $\Sigma_{casts+smears}$ m^{-2} (Figure 3.6a).

Standard tees and USGA topped tees showed a distinct bimodal seasonal activity relationship with one peak of activity in March-April (spring) and a second peak in September-October (autumn). The lowest points during the summer and the winter are significantly different from these two peaks (Figure 3.6b). Mean $\Sigma_{casts+smears}$ m^{-2} recorded on USGA topped tees increased by a factor of 10 between July-August and September-October. On standard tees earthworm activity doubled between winter (January-February) and spring (March-April). From the observations made during this study, temporary greens show the largest range in earthworm activity; maximum 2.3 ± 0.3 $\Sigma_{cast+smears}$ m^{-2}, minimum 0.3 ± 0.07 $\Sigma_{cast+smears}$ m^{-2}.

The mean earthworm activity on fairways showed a similar relationship to both types of tee construction and temporary greens. There was increased activity during the spring (March-April) followed by a decline in summer (July-August) and a resurgence in the autumn (September-October). Unlike with either of the tee constructions or temporary greens $\Sigma_{casts+smears}$ m^{-2} remained significantly higher in the winter months than earlier in the year ($p < 0.01$). This trend may be biased by the method of measuring earthworm activity as $\Sigma_{cast+smears}$ m^{-2}. The ratio of cast:smears in September-October was 0.64:1 on the fairways, however this ratio decreased to 0.17:1 during November-December. This five fold increase in detection of smears, compared to casts is probably due to smears having an increased resistance to environmental degradation during the colder, wetter winter months. This suggests that on areas of a golf course where aesthetic maintenance is minimal, assessing the biological activity of earthworms based on $\Sigma_{cast+smears}$ m^{-2} may produce some misleading conclusions. In spite of this $\Sigma_{cast+smears}$ m^{-2} is still the most suitable measure of earthworm activity on golf courses for the following reasons:

1. Although observing a smeared cast on the surface gives no indication of how long it has been there, its origin is always from earthworm activity.
2. The formation of smears is caused through land management processes, therefore the proportion of cast and smears considered as separate indicators of earthworm activity may be considerably distorted by the order in which the surfaces are sampled or by the activity of the green keepers.
3. Both casts and smears are an aesthetic challenge to the golf course, thus measuring earthworm activity by the sum of these two variables potentially results in a useful management indicator for the control of earthworms on golf courses.

a)



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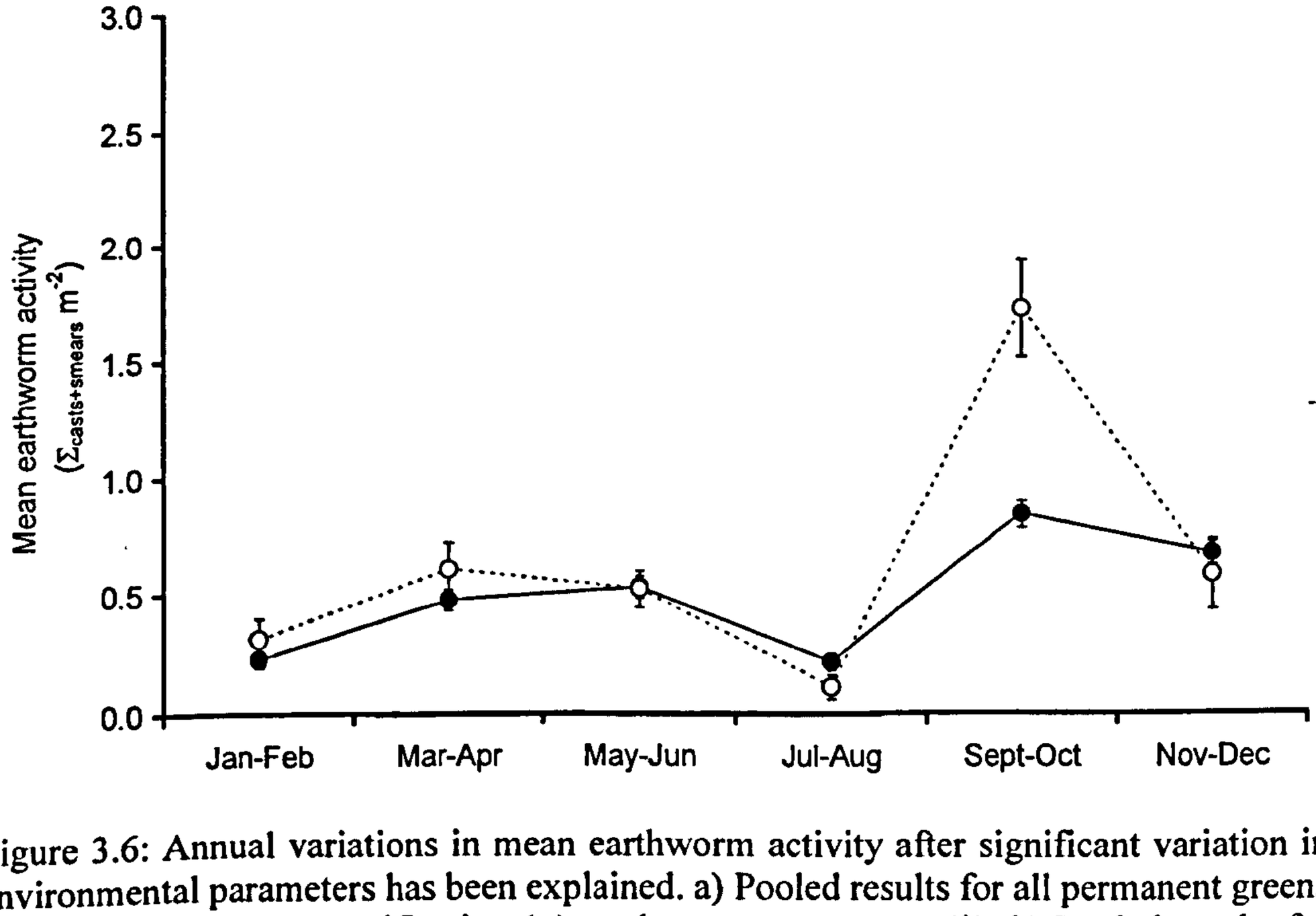


Figure 3.6: Annual variations in mean earthworm activity after significant variation in environmental parameters has been explained. a) Pooled results for all permanent greens; Standard (■), USGA specification (□); and temporary greens (◊). b) Pooled results for all tees; standard (●) and USGA topped (○). c) Pooled results for all fairways (▲). Whiskers show standard error.

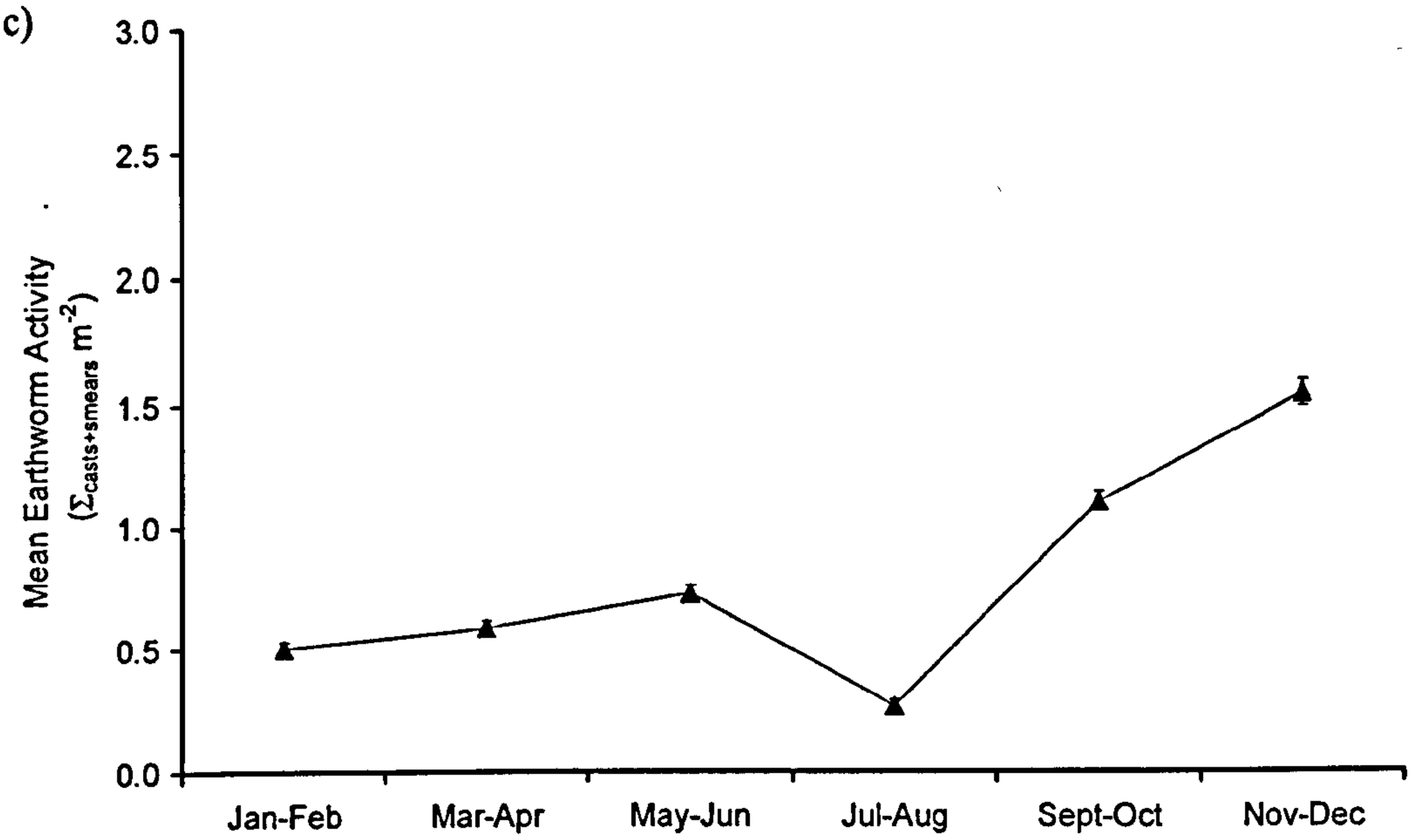


Figure 3.6: Annual variations in mean earthworm activity after significant variation in environmental parameters has been explained. a) Pooled results for all permanent greens; Standard (■), USGA specification (□); and temporary greens (◊). b) Pooled results for all tees; standard (●) and USGA topped (○) and temporary greens (◊). c) Pooled results for all fairways (▲). Whiskers show standard error.

Values for mean $\Sigma_{casts+smears}$ m^{-2} were calculated from a GZM as in Table 3.4 but environmental parameters were excluded. Multiple forward stepwise regression was then used to explore relationships capable of predicting earthworm activity from the temporally related environmental variables. Mean rainfall and mean evapotranspiration are the only significant independent variables ($p < 0.01$ in both cases; Figure 3.7). Equation 3.3 shows this relationship (adjusted $R^2 = 0.67$; $F_{(2,9)} = 12.31$; $p < 0.01$):

$$y = 0.507 + (0.348 \times R) + (-0.583 \times E) \qquad (3.3)$$

Where R = Mean rainfall during the period of estimate of activity

E = Mean evapotranspiration during the period of estimate of activity

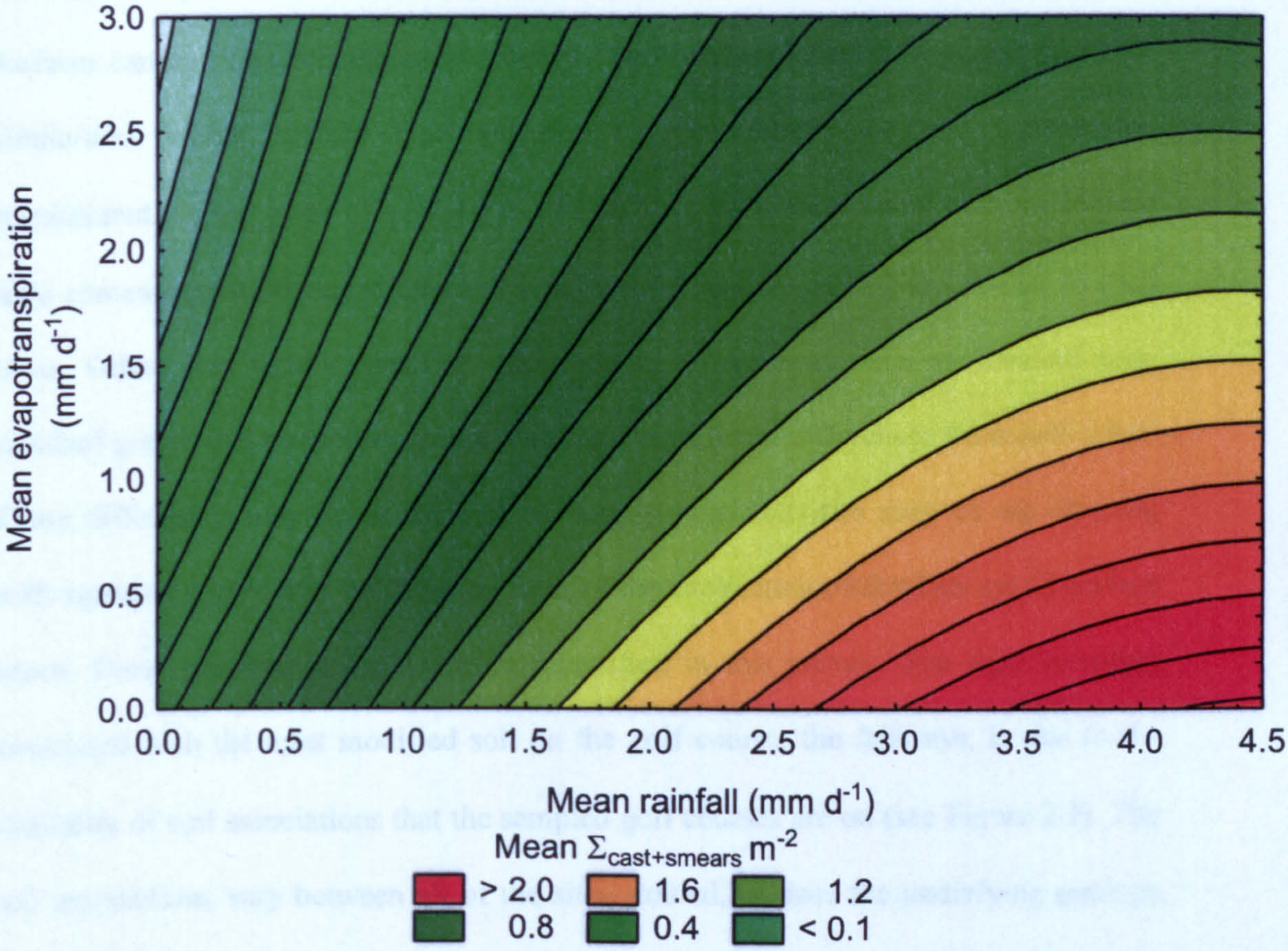


Figure 3.7: Contour plot of the variables of two significant β from multiple regression of mean $\Sigma_{casts+smears}$ m^{-2} per sampling point and all temporal environmental parameters (equation 3.3). Contours interpolated using a quadratic model.

3. 4. Discussion

The data recorded during this 24 month survey shows highly significant relationships when earthworm activity is interpreted across space, time and different areas of golf courses. The range of variability in earthworm populations both seasonally and geographically means this analysis and interpretation provides little practically applicable information with regards golf course managment. This system variability can inhibit the detection of true differences between treatments, masking any differences in the data due to the activities of the earthworms (Rombke *et al.* 2005).

All surfaces parameterised for physicochemical characteristics show that different surfaces can be characterised independently of golf course location (Figure 3.4). These similarities within surface type are due to the maintenance and construction requirements of the game of golf: surfaces that have been constructed with the highest sand contents (USGA topped tees and USGA specification greens) are similar to each other. Other, less tightly specified constructions of surfaces such as standard tees, standard greens and temporary greens also show significant differences from each other. These differences may be attributable to management activities such as top dressing with sands on greens and the differing rates of the application of fertiliser on all surface types. These parameters could not be quantified in this survey. The tight grouping associated with the least modified soil on the golf course, the fairways, is due to the similarity of soil associations that the sampled golf courses are on (see Figure 2.3). The soil associations vary between all of the sites studied, as does the underlying geology (Soil Survey of England and Wales 1983). The soils were variable but these experiments indicate that golf course management reduces this variability. The management techniques that green-keepers use to maintain the playability of these areas of the course through physical interventions must have a greater effect on the physicochemical parameters than just the textural class of the soil. This is reinforced by interpretation of the PCA loadings, non-mineral components of the soil (% total N, % total C, CEC) drawing variance associated with the fairways, and other less tightly specified areas of the golf course (Figure 3.4b).

Earthworm activity, averaged by surfaces throughout the survey and considered in relation to the physicochemical make-up of each of these surfaces shows interesting

relationships. Significantly different associations formed in the physicochemical PCA have correspondingly different earthworm activity associated with them (Table 3.2 and Figure 3.4). From the surfaces that are found at all courses the fairways have the greatest earthworm activity (mean $\Sigma_{casts+smears}$ m^{-2}). While the fairways receive relatively little attention with regards to earthworm cast reduction there are some incidents of green-keepers using chemical controls, such as carbendazium (see Chapter 1.3) on some fairways (*personal observations*). As earthworm control methods are in place for all surfaces on the golf courses studied the recorded mean annual earthworm activity of 0.73 $\Sigma_{cast+smears}$ m^{-2} can be considered as a maximum acceptable level of earthworm activity per year. This statement can also be extended to all the other surfaces in the study (e.g. see Table 3.2).

The survey indicates that USGA topped tees have more than a three-fold increase in earthworm activity when compared to USGA specification greens. A similar trend is seen with standard construction tees and greens. The low earthworm activity recorded on the greens is attributed to the green-keepers giving the highest priority with regards to presentation of these surfaces. With a bimonthly sample period it was not possible to test the impact of any chemical application by the green-keepers on the earthworm community. The few chemicals that are currently cleared for application to amenity turf have very short periods of effect on earthworm activity and thus at this time resolution it is impossible to draw any conclusions about their use (Perris 1996b). More recent research of other horticultural systems suggests that the effect of compounds such as carbendazium have only an intermittent effect on earthworm biology (see Chapter 1.3) and have the greatest effect where the population of earthworms is already low

(Reinecke and Reinecke 2006). The use of this chemical on all golf courses surveyed (*Personal communications; Golf course managers*) must therefore contribute to the low level of earthworm activity observed on the greens.

The tee areas, which share many soil characteristics with greens, have greater earthworm activity as they are not as highly managed. An increase in earthworm activity can be attributed to an increase in human traffic in this area of the course: each tee is one of the few areas that all players will visit at each hole, foot traffic is likely to be more concentrated at the tee, while it is assumed that each player will also visit each fairway and green on a golf course they are less likely to stand in the same place as other players. The resultant is greater soil compaction on the tee. Work carried out in maize fields shows that areas of greater compaction, i.e. tractor wheelings have almost a 50% increased level of earthworm cast formation when compared to adjacent, non compacted inter-crop areas of the field (Binet and Le Bayon 1999). In soils with a high sand content, such as the USGA topped tees, vibrations and compactions caused by this increase in human traffic is likely to result in earthworm burrow collapse. It may also cause soil particles to fall back into earthworm burrows after they have been deposited on the surface. These two causal mechanisms are suggested where the proportion of sand in the soil is high. The weak cohesive and adhesive forces between the sand particles means that both casts and burrows can be broken with little force (Brady and Weil 1999). This increases the frequency that earthworms must excavate their burrows in response to habitat destruction, and so recorded levels of mean $\Sigma_{cast+smears}$ m^{-2} are greater on this surface type.

3. 4. 1. Earthworm activity in relation to physicochemical parameters

The result of the analysis of the 50,404 quadrats observed during this two year study means that Hypothesis 3.1 is accepted (*The activity of earthworms on different surfaces of golf courses varies with the surface construction due to the differing physicochemical parameters related to each surface*). Soil physicochemical parameters had a strong relationship with earthworm activity, however it is considerably more complex than a univariate interaction. Predictions are not based a single factor, but many inter-related components. The physicochemical parameters measured in this study showed % total organic carbon, C:N, % sand, CEC and % silt were the most important in describing mean $\Sigma_{casts+smears}$ m^{-2}. These variables contribute to an un-validated prediction equation for earthworm activity on parkland golf courses (Table 3.3 and Equation 3.2). This model shows great potential as a tool for golf course architects (and those designing other sports surfaces) to select soils that will have inherently low earthworm activity. The model highlights similar significant physicochemical components to other work carried out on agricultural land. A spatial earthworm survey, using formalin extraction, on agricultural sandy loams in Germany, shows that patterns of earthworm distribution can be related to soil pH, % total organic carbon and % total nitrogen, % clay content and C:N ratio, using the same forward stepwise multiple regression technique. No measurements of CEC were made in their survey (Joschko *et al.* 2006). pH does not account for any variance in the golf course model, due to the narrow range of pH found on the surveyed courses (mean pH = 6.3, ± 0.15 at 95% confidence) where it is included in the model of Joschko *et al.* (2006). The soil variables outlined above can be associated with the quality and quantity of the turf growth. The relationships interpolated in Figure 3.5, between the two variables with greatest significance to earthworm activity (% total organic carbon and C:N) can be interpreted in this way.

Where there are the best soil conditions for grass growth, there is the highest earthworm activity. This is due to an increase in food availability. Similar trends relating nutrient addition and site management to the size and structure of earthworm communities have been described in upland pastures (Cole *et al.* 2006). The nutrient balance required to achieve the optimal growth rate for grass in the pastureland (the same as those described by the bottom right quarter of Figure 3.5) were related to the highest rates of earthworm activity. Measurements in this study were only during the autumn. A microcosm experiment carried out by Lowe and Butt (2002) observed a similar relationship, showing that greater densities and activities of earthworms could be correlated to greater concentrations of soil organic matter. The mechanism behind the results displayed by this work may not be as simple as these workers suggest. Postma-Blaauw *et al.* (2006) have shown that both carbon and nitrogen cycling is affected by the diversity of the earthworm population present. Different community structures are capable of actively changing the total organic carbon content of the soil and altering the processes of N mineralization. The size and structure of the earthworm community will be reflected in the mean $\Sigma_{casts+smears}\ m^{-2}$. It is unlikely that the differences in soil chemistry of different treatments on the golf courses is as a direct result of different earthworm communities due to the range of other biocide and fertiliser inputs to the system. However, different availabilities of both carbon and nitrogen on these surfaces may have compounded the community differences: soils which have large available pools of carbon and nitrogen have the greatest earthworm activity. Some workers have shown contradicting findings: earthworm activity measured in both savannah and pasturelands in Colombia, with different soil management techniques, were shown not to be significantly different (Jimenez *et al.* 2001). This suggests that any predictions that

Equation 3.2 is capable of may be limited to parkland golf courses on similar soil associations to the ones measured here.

3. 4. 2. Earthworm activity in relation to seasonal variations

Temporal trends in earthworm activity elucidated from the data indicate that for the surfaces studied here Hypothesis 3.2 is accepted, with some exceptions (*Earthworm activity increases during the spring and autumn months on all areas of golf courses relating to the life cycle of earthworms*). On all tee constructions, fairways and temporary greens two peaks of earthworm activity are seen, one in spring and one in autumn. These follow the current models and understanding of earthworm life cycles; Figure 3.6b & c (Lee 1985). Laboratory experiments designed to follow these trends in activity have shown that there are large inter-individual variations. Increased variability in activity is also seen where earthworms experience greater environmental stresses. In treatments of increased thermal and soil-water stresses, the mean time (days) for earthworms to reach sexual maturity is longer. These earthworms are also less active, both sexually and generally by the time they reach this age. Food supply and chemical toxins are also cited as significant factors affecting the earthworms life cycle (Jager *et al.* 2006). The range and variability in these environmental parameters on golf courses is significant: the potential stresses are elevated on the greens. The more intensive management (and so increased earthworm stress) of the green accounts for the limited activity on this surface at all time of the year (Figure 3.6a). The level of earthworm activity ($\Sigma_{casts+smears}$ m^{-2}) is therefore low on these surfaces as years of maintenance and chemical control has suppressed the endemic earthworm population.

Other temporal earthworm populations studies, in arable fields, have been shown to relate to earthworm population dynamics to soil moisture (Schmidt and Curry 2001). Earthworms from these experiments were enumerated by hand sorting soil cores taken randomly from experimental fields at bimonth intervals for two years. The highest populations were recorded in the autumn, during the same periods that the highest $\Sigma_{casts+smears}$ m^{-2} are recorded from golf courses. In the same field experiment a smaller peak in earthworm population is also seen during the spring (Figures 3.6b). Schmidt and Curry (2001) also recorded the lowest populations in the summer. Similar trends in activity have also been reported based on hand-sorting of Canadian soil samples (Tomlin *et al.* 1992). Earthworm activity dynamics from these systems are again related to soil moisture: more earthworms were recorded in the autumn and spring than the summer. The widely reported increases in activity of earthworms during the spring and autumn have been related to earthworm life cycles and reproduction. Earthworms become sexually active in the spring, producing cocoons which remain in the soil during the summer and hatch in the autumn. Hence there is a more activity in the spring and autumn (James 1992).

3. 4. 3. Earthworm activity in relation to meteorological variations

Meteorological data collection was limited to records made in Silsoe, Bedfordshire. However clear a relationship is evident and Hypothesis 3.3 is accepted (*Annual variations in earthworm activity are related to the local environmental meteorological changes as earthworms respond to the changing gradients in these environmental parameters*). In the most simplistic terms the greatest earthworm activities were recorded when there are better seasonal conditions for plant growth during the spring and autumn (Figure 3.7). Similar effects have been recorded in Spain with studies

focused on the endogeic species *Hormogaster elisae* by Valle *et al.* (1997). In Spain, where an annually cyclic pattern of rainfall, with a period of rainfall, followed by dry conditions, then rainfall then dry again is common. With this bimodal rainfall a corresponding bimodal earthworm activity was recorded, and attributed to earthworms burrowing deeper into the soil to keep themselves within tolerable parameters of soil moisture. The relationship between mean rainfall and evapotranspiration and earthworm activity ($\Sigma_{casts+smears}\ m^{-2}$) on golf courses can be related to soil moisture and interpreted in a similar way to these findings with for *H. elisae.*

In agricultural grassland systems, where mowing is a more infrequent event than on golf courses, the food availability to the earthworms at all times of year is the most important factor driving earthworm activity and life cycles (Daniel 1992). By comparison to temperature controlled laboratory based systems Daniel (1992) also suggests a relationship between temperature and growth rates (analogous to activity) with higher temperatures significantly reducing estimated earthworm lifespans (50 weeks at 15 °C; 127 weeks at 9 °C). It was not possible to determine soil temperature at any sample events during the field experiments and thus similar interpretations can not be made. Other work suggests that the population structure and therefore casting activity is effected by the rate and frequency of irrigation in prairie grass (James 1992). A very similar effect is recorded here with respect to earthworm activity, which was increased as mean rainfall increases (x axis, Figure 3.7).

Extending this work to studies of natural and semi-natural grasslands could provide interesting information as to how wide-ranging the applicability of this model might be as a potential measure of soil health. A further study, with an even greater level of

resolution in terms of both earthworm cast surveys and environmental factors measured may lead to a more accurate prediction of earthworm activity on each specific area of the golf course. Analysis at a very fine scale may be able to relate water balance models for specific golf course surfaces in relation to earthworm activity. This could be of great use to golf course managers for the timing and duration of course irrigation.

Chapter 4: Development of the mustard extraction technique in order to representatively assess earthworm community size and structure on fine turfgrass.

4. 1. Introduction

There are inherent problems with making surveys of earthworm species, as to make an unequivocal assessment of populations the technique used must be invasive to the earthworm's natural environment. Darwin (1883) noted that earthworms found in his samples of soil were chemotactic, i.e. they moved in predictable ways in response to chemical gradients. This behavioural trait can be used to force worms to the surface by applying an irritant chemical at the surface. The other method that Darwin initiated was hand sorting soil volumes for earthworms. Both of these sampling approaches have been developed further such that ISO and British Standard protocols have been developed, *viz.* BS7755-4.2.3:1999 and ISO 11268-3:1999 (ISO 2000).

4. 1. 1. Destructive sampling

It is widely acknowledged that hand sorting is the most representative method for sampling earthworm communities (Edwards and Lofty 1977; East and Knight 1998; Fox 1998; Chan and Munro 2001). It is also the most destructive approach, since it requires a complete disintegration of a representative soil volume. Handsorting can be carried out in the field but it is a time-consuming process (at least sixty minutes per 0.2 x 0.2 x 0.2 m sample (8 L) – although greater accuracy requires more thorough and therefore slower sorting (*M. Pawlett; personal communications*). A skilled operator can locate and isolate worms of a fresh weight as low as 0.2 g and detect earthworm cocoons. The cocoons of some earthworm species are sufficiently distinct to allow them to be identified to species level (Edwards and Lofty 1977).

The greatest problem with the hand sorting method is the length of time that is required to produce reliable results. Work has shown that the efficiency and speed of hand sorting can be increased in a number of ways. Several different systems have been developed of soil washing (Satchell 1967). This process uses water to either to make a soil solution from the sample, or is sometimes applied under pressure to disintegrate the soil. Both of these methods can damage earthworm specimens and complicate species identification, but increase the speed of earthworm recovery and are capable of recovering smaller specimens. In some cases an aqueous solution with a specific gravity of greater than 1.0 g^{-1} cm^3 is used. This means that earthworms with a specific gravity of less than 1.4 g^{-1} cm^3 will float to the surface of the soil solution. This is commonly achieved using an $MgSO_4$ solution. This technique can also be used to recover cocoons. The efficiency of these washing methods depends on the soil texture because of cohesion between soil particles in soils with high clay contents which prevent adequate disintegration.

Sampling for earthworms in sports turf is challenged by the requirement that only non-destructive samples can be taken; the aesthetic presentation of golf courses is of extreme importance. Destructive, or sampling that damages the turf, is unacceptable. This coupled with the high land value – the most prestigious of golf greens (USGA specification constructions) cost in excess of £250 m^3 in materials alone (*P. Jones, golf course architect; personal communications*) – means that green keepers are understandably unwilling to permit disruptive or destructive sampling practices.

4. 1. 2. Behavioural sampling

Behavioural responses of earthworms have been exploited for sampling and in general these methods are either non-destructive to the sampling surface or cause very limited physical damage to the soil. Raw (1959) piloted a means of chemical extraction using formalin. Formalin is an irritant to earthworms but also can be used for fixing samples for later analysis. It does this by forming cross linkages between proteins and the oxygen atom in the compound. This method is particularly effective for fixation as these cross-linkages preserve the protein structure.

A 0.2% aqueous solution of formalin is applied to the soil and within minutes earthworms appear at the surface, in an attempt to escape the irritancy of the chemical. This method is not widely used anymore in the UK because of Control of Substances Hazardous to Health (COSHH) regulations. Formalin is a carcinogen and highly toxic to the operator, earthworms, and general soil system. Unless worms that emerge are washed thoroughly they will die, which makes identification considerably more challenging. There are reports in the literature of the phytotoxic effects of formalin to sports turfs and the general soil system thus making it particularly inappropriate in the context of the studies in this thesis (Gunn 1992; Cook *et al.* 1997; Eichinger *et al.* 2006).

Another chemical shown to have a similar expellant effect on earthworms, but which is considerably less toxic to both earthworms and operator is allyl isothiocyanate (Zaborski 2003). This compound is found in mustard (*Brassica nigra* L.) and gives the plant's seeds their 'hot' flavour; it is a potent irritant to mucus membranes (Salisbury and Ross 1992). Allyl isothiocyanate is an alkaloid that is produced through the natural breakdown of glucosinolates in mustard seeds. The mustard extraction was piloted as

an ecologically benign method by Gunn (1992) in the search for a more time efficient method than hand sorting. He showed a hyperbolic relationship between the number of earthworms extracted and increased concentration of a solution of 'English Mustard' paste. Gunn also noted problems with making high concentration solutions as the mustard paste would not remain in solution. Similar problems have been reported by other workers (East and Knight 1998; Chan and Munro 2001). More recently research has shown that a suspension of mustard powder in water is most effective (Fox 1998; Lawerence and Bowers 2002) producing an estimated 0.2% aqueous solution of allyl isothiocynate (using 6 g mustard powder in 1000 mL of water). Lawerence and Bowers (2002) determined that this method can account for 98% of the total worm biomass of a soil sample and 83% of species when compared to hand sorting. The methodology for chemical expellance requires large volumes of water. The British Standard protocol (BS 7755-4.2.3: 1999) suggests the use of 10 L over 0.25 m^2 for both the formalin and mustard methods.

The use of commercial (catering) mustard powder introduces other problems. Mustard powder is an inconsistent product, with some containing less than 50% mustard flower (e.g. Brakes English Mustard: mustard flour, ≈49%; wheatflour, ≈51%; colour (E100), < 0.05%; pepper, < 0.05%). This problem can be reduced by using "high-quality" mustard such as Colman's English Mustard of which the listed ingredients is exclusively mustard flour. The growing season, source and variety of mustard seed used to produce this flour is not detailed on products and these factors will have an effect on the concentration of the alkaloids in the flour. This is also likely to vary between batches produced at the mill (Chan and Munro 2001; Zaborski 2003). The use of purified allyl

isothiocyanate has been used by some workers, however like formalin it is hazardous, controlled by COSHH in its undiluted form (Zaborski 2003).

Other chemicals have also been trialled, such as potassium permanganate and household detergents. These have been shown to be both ineffective and toxic to earthworms. Experiments with detergent showed high concentrations of compounds (e.g. 30 mL L^{-1}) were toxic to earthworms extracted; all worms that appeared at the surface had swollen clitella and 70% were dead within 24 hours (East and Knight 1998). In trials on sports turf, potassium permanganate was shown to have phytotoxic effects on the turf and underestimated the number of worms in the sample area (Binns *et al.* 1999).

Earthworms will react to an electrical current. When electricity is passed through the soil it can make them come to the surface (Rushton and Luff 1984). This characteristic has been exploited to develop a sampling technique known as electromigration. An apparatus, normally with eight electrodes in a circular arrangement is hammered into the soil to an appropriate sampling depth and an alternating current is applied (Rushton and Luff 1984). Earthworms that come to the surface in the ring of electrodes are taken as the sample. There are several problems with using this method for repeated samples of earthworms because there are a number of variables that are hard to control. One of the principle variations between sampling sites is the soil conductivity. This factor is dependent on both soil water content and soil type. These factors affect the concentration of salts in solution that are responsible for carrying the current (Brady and Weil 1999). This change in resistance with varying water content means that the effective sample volume is liable to change. An extensive soil analysis is required to

recalculate the volume each time the analysis is carried out. Another major problem with this sampling method, noted by Rushton and Luff (1984) is that the method can force worms down the soil profile to escape the electrical current. This means that the deepest burrowing anecic earthworms may be under-represented in the sample. There is also the problem of generating the voltage and current required for this sort of extracting (normally 240 V at 5A) in the field, an alternating current generator being required. Working with this sort of apparatus in the field also presents a range of safety considerations. A further problem with this method is that different potential differences in the electrodes will affect worms in different ways. This varies with the total surface area of the earthworm. Larger earthworms, with the greatest surface area need to have a larger potential difference between electrodes to have an effect. With this higher electrical power rating earthworms with a smaller surface are potentially causing death by electrocution. A range of currents cannot be effectively used because a small potential difference will cause large earthworms to migrate down the soil profile, out of the sample volume (Thielemann 1986).

4.1.3. Combined methods

Due to the advantages and disadvantages presented by both hand sorting and chemical expellance an optimal method is to combine the two (Springett 1981). Soil cores are taken for hand sorting in the lab and a chemical expellant then applied to the bottom of the pit left after the core has been removed. In this way optimal recovery is achieved. Edwards and Lofty (1977) suggest that the most effective method for population surveys is to take pit samples for hand sorting and chemical expellance. If this is done in the ratio of 1:2 (hand sorting:expellance) representative and consistent results can be obtained.

4. 1. 4. Methods in development

Research by the United States Department of Agriculture has demonstrated that pest insects can be located in the soil of horticultural potted plants using acoustic systems. This application has been used as quality control for pest species in horticultural nursery stock plants. By putting a sensitive microphone into the plant pot, movements can be detected in the soil (Mankin and Fisher 2002). The same researchers have used a similar technique to detect insect larvae pupating in the stems of plants (Mankin *et al.* 2000). This work highlights two important problems if this approach were to be developed as a field method for sampling earthworm populations: The propagation of a detectable sound wave in soil, made by an insect (or other soil invertebrate) is between 0.05 and 0.3 m depending on soil conditions. This means that it would be very challenging to accurately and repeatedly sample adequate volumes of soil. Both papers by Mankin *et al.* (2000; 2002) make the suggestion that background noise plays a significant role in the efficiency of this method, the best results being seen in quiet experimental greenhouses, with the doors shut. It is uncertain because of this as to whether the method could be transferred to an environmental context such as golf courses were there would be a relatively high background noise. Potentially a method like this could be used for non-destructive enumeration of the total earthworm population within the soil. If these problems could be overcome, further research work would be needed in order to make distinctions between the species found. The concept has potential but would require extensive research.

A competition, the 'World Worm Charming Championships' has been held annually since 1980 in Nantwich, Cheshire, UK (Figure 4.1). The aim of this competition is to raise as many worms as possible out of a 3 x 3 m area of turf (in this case a school

playing field) in 30 minutes. In the competition, digging and the use of chemicals are not permitted. The "world record" as of 2006 is 511 worms; approximately 57 earthworms m^{-2} (IFCWAP 2006)[6]. The most effective method of getting worms out has been shown to be "twanging". In this technique a 4-prong garden fork is put into the ground at a depth of approximately 15 cm and slapped repeatedly. There is little about the sampling of earthworms by this method either in the peer-reviewed or 'grey' literature. Based on observations made at the 26th World Worm Charming Championships and during pilot studies made within the grounds of Cranfield University at Silsoe, the most probable causal mechanism for earthworms migrating to the surface is in response to habitat destruction, or disruption. The development of this approach is inappropriate for sampling earthworms from sports turf as it results in considerable damage to the turf surface.

Figure 4.1: The 26th World Worm Charming Championships, 24th June 2006. Nantwich, UK.

[6] 26th World Worm Charming Championships (2006): Bartlett, Ritz and Harris – 13th place out of 144 teams, 49 earthworms.

4. 1. 5. Sample storage

Earthworms can be kept alive in oxygenated water for up to several months or stored in soil samples at temperatures between 5 to 10°C (Lee 1985). There are a range of ways of fixing samples. Formalin or 70% alcohol/industrial methylated sprits will both fix and preserve earthworms (Edwards and Lofty 1977). It has been noted that this can do some damage to cellular structure and to maintain the quality of the samples the preservative must be changed at regular intervals (Edwards 1996). Fixing earthworms in this way causes discoloration and in many cases masks diagnostic features to speciation. The least damaging way of fixing earthworms, especially if dissection is required, is to submerse specimens in a water bath at 50°C for 20 minutes (Lee 1985).

4. 2. Evaluation of the efficiency of mustard extraction in estimating total earthworm population

Mustard extraction is widely used, and an international standard is defined (ISO 2000). However, no formal assessment of its effectiveness with UK earthworm species has been carried out. In this study the extraction efficiency of mustard solution was evaluated under the hypothesis:

Hypothesis 4.1. A representative proportion of the extracted earthworm community will be expelled when mustard solution is applied to a defined surface area of turf.

In order to apply mustard solution to commercial golf turf it is essential that it is established that no damage will be caused to the grass. Plant phytotoxicity of mustard solution has not previously been addressed because ISO 11268-3:1999 is principally concerned with sampling in contaminated land areas. It was essential that a guarantee could be given to golf course managers that any putative application of mustard solution

to turf, would have no cosmetic implications to turfgrass. The following hypothesis was tested to underwrite any such guarantees.

Hypothesis 4.2. When a mustard solution at standard concentration or at four times the normal concentration, is applied to simulated golf course surfaces there will be no effect to turf health.

4. 2. 1. Materials and Methods

Zabroski (2003) determined that allyl isothiocyantate is the active alkaloid in mustard that causes the chemotactic response in earthworms. He also suggests that there is variation in the strength of allyl isothiocyanate in mustard powders from different suppliers and batches from the same supplier due to it being based on a natural product and thus is linked to the maturation of the mustard plant (*Brassica juncea*). To eliminate variation in the concentration of allyl isothiocyantae in the mustard flour, a batch of 40 kg was acquired from Colman's Mustard (Norfolk, UK), and used throughout the studies in this thesis.

Ideally, these trials should be tested on golf turf. However it was not possible to locate a golf course where destructive sampling could be carried out and therefore experiments were conducted in a 200 m^2 area of pasture land at Silsoe College farm, Bedfordshire. The sward cover was mown to a height 50 mm for the preceding two months to simulate a golf course fairway. The soil type was classified as sandy loam using the Bouyocous method; soil pH was 6.70 ± 0.17; CEC was 24.4 ± 1.68 cmols kg^{-1}, see Chapter 2.4 for details of methods. Sampling was conducted on the 31st March and 1st

April 2005. Investigations of phytotoxicity to turf grass plants was carried out as a pot trial in the experimental greenhouses at Cranfield University at Silsoe.

4.2.1(a) Mustard extraction

Mustard extraction relies on the chemotatic movement of earthworms in response to irritants in mustard solution, however earthworms are a terrestrial species so may have a chemotatic response to water alone. These factors were considered in the experimental design. Three treatments were used:

- No surface application (designated Control).
- Water only treatment (designated Water).
- Mustard solution at 6 g L^{-1} m^{-2} (designated Mustard).

Each treatment was applied using the protocol of ISO 11268:1999 (ISO 2000). Ten litres of solution was applied over a 0.25 m^2 area defined using a circular steel infiltrometer (0.28 m diameter, 3 mm wall thickness) pressed approximately 10 mm into the soil surface to prevent lateral seepage. Seven replicates of each treatment were applied, using a randomised design. The assessment assumed a uniform distribution of earthworms across the whole sample area.

Treatments were applied to the surface within the ring infiltrometers and all expelled earthworms were collected over a 30 minute period following application. Subsequent to this the soil below the ring was excavated to a depth of 0.2 m. The soil from each replicate was hand sorted in the field to recover residual earthworms in the soil. Earthworms recovered from each treatment were categorised as expelled (E), residual (R), or total (T); Figure 4.2. The total population is defined as the sum from both hand

sorting and surface extraction. The control treatment represents an unbiased sample of the extant earthworm community. Earthworms were stored in oxygenated water at 2 ± 0.5°C prior to speciation. Adult earthworms were identified by the presence of a clitellum. Earthworms without a cilellum where identified were possible and recorded as juveniles.

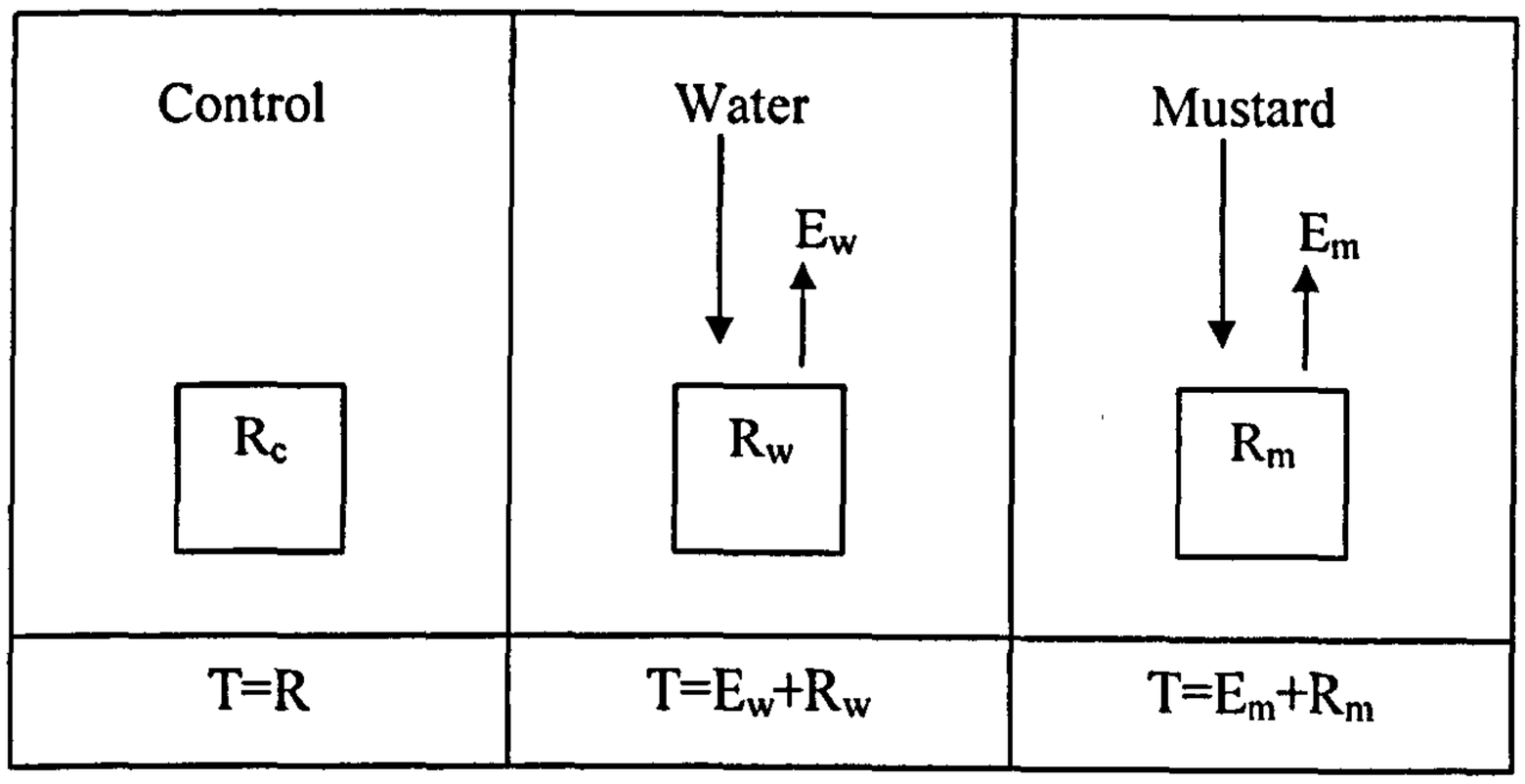


Figure 4.2: Schematic diagram of experimental design and notation. E = expelled; R = residual; T = total; c = Control; w = Water; m = Mustard.

Comparisons were made based on earthworm frequency and Simpson's diversity indices, i.e.

$$Simpson's\ D = \frac{1}{\sum_{i=1}^{s} p_i^2} \tag{4.1}$$

$$Simpson's\ E = \frac{1}{\sum_{i=1}^{s} p_i^2} \times \frac{1}{s} \tag{4.2}$$

Where: s = total number of species in the community

p_i = proportion of S made up of the *i*th species

Simpson's D informs about the diversity of the system, values may be any positive number with greater values representing wider diversity. Simpson's E relates to

equitability describing the spread of species in the ecosystem, values range from 0 to 1. A value of 1 denotes total equitability (Vadermeer and Goldberg 2003). Simpson's diversity indices were selected over other diversity indices due to increased accuracy in the representation of community diversity when the sample size is less than 1,000 observations (Magurran 2004).

Community structures were compared using principal component analysis (PCA) and one-way analysis of variance (ANOVA) of the resultant components. All analysis was carried out using Statistica 7.1 (Statsoft Inc. 2005).

4.2.1(b) Phytotoxic effects of mustard solution to turfgrass

Turf grass growth trials were carried out in the experimental greenhouses at Cranfield University at Silsoe, Bedfordshire. Turf was grown under artificial lights on a 16:8 h, light:dark cycle between February and April 2005. Two different golf green construction materials were used: a sandy loam soil (72% sand; 16% silt; 12% clay) and USGA rootzone (95% sand; 5% silt and clay). Three treatments were applied to each construction material.

- Water (designated Control).
- Mustard solution, 6 g L^{-1} m^{-2} (designated Normal)
- Mustard solution, 24 g L^{-1} m^{-2} (designated High)

The High treatment was used to describe the effects from any potential magnification and to parameterise any possible risks to the turfgrass. A concentration of 24 g L^{-1} m^{-2} adopted since this was effectively the maximum concentration that could be attained.

Six replicates were used per treatment on each construction material. Each turf was grown in a 2.9 L plant pot (170 mm diameter). All pots were packed to a dry bulk

density of 1.5 Mg m^{-3}. An 80:20 *Festuca* spp :*Agrostis* spp grass seed mix was used for all replicates. The replicates were be arranged in a 6 by 6 Latin square (Figure 4.3). A Latin square design was used as a lighting gradient was present from right to left of Figure 4.3 and a gradient of air movement, between doors, was present from top to bottom of the greenhouse.

USGA Control	Sandy Loam High	USGA Control	Sandy Loam High	USGA Control	USGA Normal
Sandy Loam Control	USGA Normal	USGA High	USGA Control	Sandy Loam Normal	USGA Normal
Sandy Loam High	Sandy Loam Control	Sandy Loam High	Sandy Loam High	USGA Control	Sandy Loam Normal
USGA Normal	Sandy Loam Normal	USGA High	USGA High	Sandy Loam High	Sandy Loam Normal
USGA Normal	USGA Normal	Sandy Loam Normal	Sandy Loam Control	Sandy Loam Control	USGA High
USGA High	USGA Control	Sandy Loam Control	USGA High	Sandy Loam Control	Sandy Loam Normal

Figure 4.3: Treatment layout in distribution of phytotoxicity experiments.

Damage to turf was assessed by visual inspection and dry weight yield of clippings. Both assessments were made every 7 days for 28 days. Visual assessments were recorded using a Nikon Coolpix 4500 digital camera from an objective height of 1 m. All image manipulation was carried out using Adobe Photoshop Elements (2001). Replicates for comparison were selected at random but the same replicate was used at each time point for consistency thought out. All images were taken prior to grass clippings being taken. The turf grass was clipped to a height of 5 mm above the soil

surface and all clippings were collected and dried for 18 h at 105 °C to establish grass dry weight. One-way ANOVA was carried out on these data. Visual analysis was made in a qualitative manner comparing replicates from each treatment on each construction material.

4. 2. 2. Results

A total of 1117 earthworms were recovered from all treatments in the experiments, of which 39.7% were adults (see Appendix III). Seven different species were represented with both adults and juveniles apparent from all but *Lumbricus festivus*, where only adult earthworms were found; 3.9% of juveniles were not identifiable (Table 4.1).

Table 4.1: Frequency and species distribution of earthworms recovered from all sampling points and treatments.

Species	Adults	Juveniles	Total
Lumbricus terrestris	73	45	118
Aporrectodea rosea	168	310	478
Aporrectodea caliginosa	43	127	170
Lumbricus rubellus	48	44	92
Allolophora chlorotica	46	76	122
Lumbricus castaneus	60	46	106
Lumbricus festivus	5	0	5
Unidentifiable	0	26	26
Total	443	674	1117
% of Total	39.7	60.3	

The mustard treatment was the only method to expel earthworms to the surface, and 35.7% of the total population emerged (Table 4.2). There was no significant difference in the total number of earthworms between any of the treatments ($p = 0.14$).

Table 4.2: Mean number (with standard error) of expelled, residual and total earthworms from each treatment (n = 7). Letters indicate heterogeneous groups using Fisher LSD.

Treatment	Expelled (E)	Residual (R)	Total (T)
Control	0.0 a	67.9 ± 7.61 b	67.9 ± 7.61
Water	0.0 a	43.0 ± 8.57a	43.0 ± 8.57
Mustard	17.4 ± 2.54 b	31.3 ± 8.57 a	48.7 ± 9.32
F	46.95	4.87	2.22
p	<0.01	0.02	0.14

Simpson's D showed significant differences between earthworms from all expellance treatments and the extant earthworm population (p = 0.04; Table 4.3a). Simpson's E showed no significant differences between treatments (p = 0.380). The Mustard treatment showed a significantly smaller Simpson's D than the other two treatments in the residual soil volume (p = 0.02), however Simpson's E showed no significant difference (Table 4.3b). Simpson's E for the total earthworms from all treatments indicates that the population in the Mustard treatment was less equitable than the other two treatments (p = 0.03).

In the analysis of community structure the first three principal components (PC) accounted for 71% of the variation between samples, however only PC1 showed significant treatment effects (Figure 4.4a). The loading values of PC1 showed that juvenile *Ap. rosea* and *Ap. calagonsis* were heavily negatively loaded while adult *L. rubellus*, *Ap. rosea* and *L. terrestris* were heavily positively loaded (Figure 4.4b).

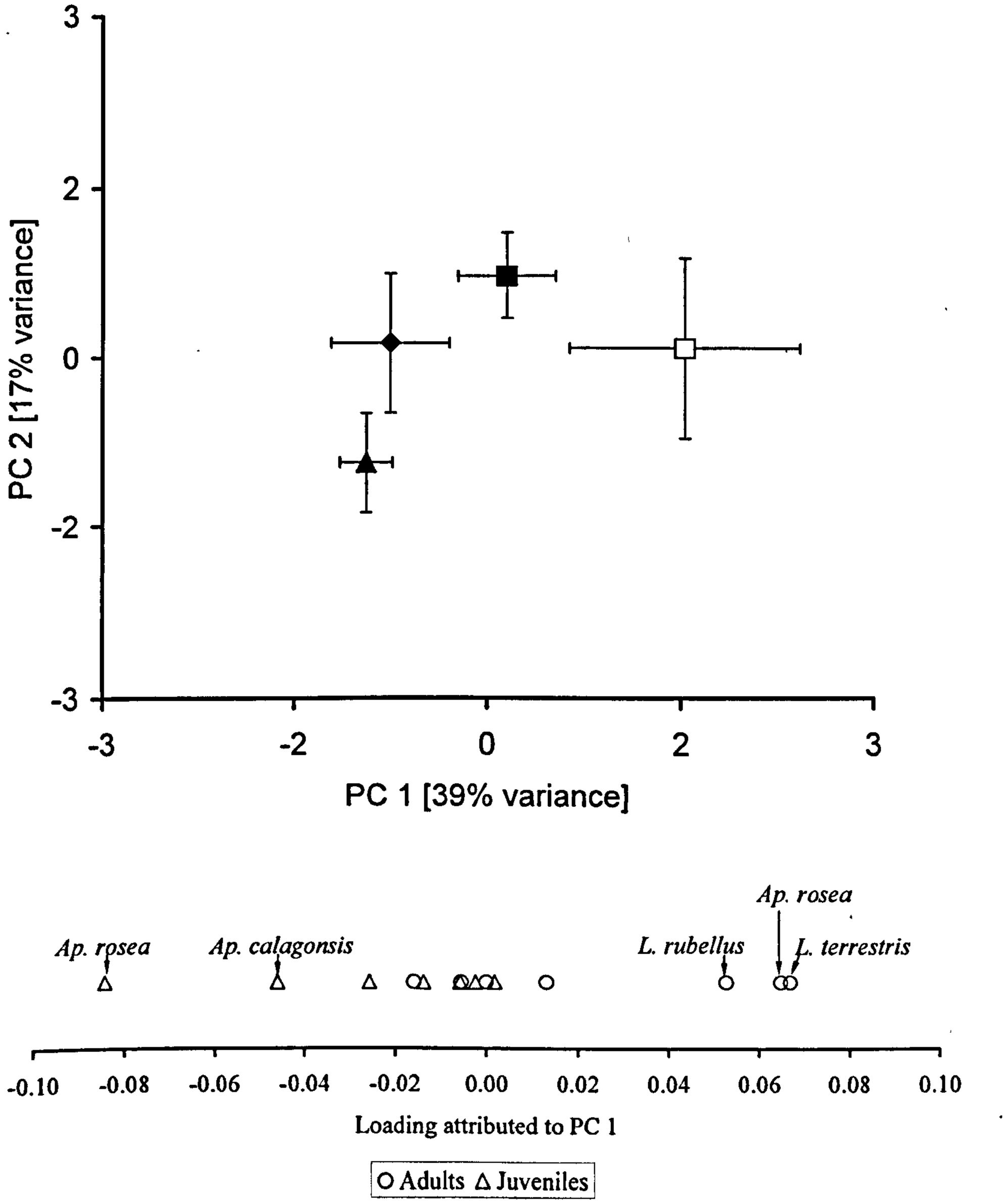


Figure 4.4: Principal components (PC) analysis of earthworm communities identified by different extraction procedures. a) Mean co-ordinates of PC1 and PC2 with respect to total communities identified in treatments; Water (♦); Control (▲); Mustard (■) and extracted community in Mustard treatment (□). Whiskers show standard error of the mean, n = 7. b) Projection of loading values associated with significant differences in PC 1.

Table 4.3: Species diversity indices calculated for each fraction of earthworms recovered. a) Mean (with standard error) Simpson's D; b) Simpson's E. (n = 7). Letters indicate heterogeneous groups using Fisher LSD.

a)

Treatment	Simpson's D		
	Expelled	Residual	Total
Control	0.00 a	3.62 ± 0.25 a	3.62 ± 0.25
Water	0.00 a	4.19 ± 0.32 a	4.19 ± 0.32
Mustard	3.12 ± 0.26 b	2.77 ± 0.40 b	3.15 ± 0.28
F	141	4.71	3.35
p	< 0.01	0.02	0.06

b)

Treatment	Simpson's E		
	Expelled	Residual	Total
Control	0.00 a	0.65 ± 0.03	0.65 ± 0.03 a
Water	0.00 a	0.65 ± 0.03	0.65 ± 0.03 a
Mustard	0.72 ± 0.06 b	0.56 ± 0.05	0.55 ± 0.03 b
F	166.48	2.08	3.99
p	<0.01	0.15	0.03

The dry weight of clippings from all treatments showed variability consistent with seasonal variation in temperature. There was significant difference in the dry weight of clippings from all treatments, including Control, between 7 and 14 days post application; $p < 0.01$ (Figure 4.5).

Dry weight of grass collected on the sandy loam treatments showed no significant difference from the control treatment at any time (Table 4.4a). The USGA rootzone treatments, however, showed significant less dry weight of clippings at 14, 21 and 28 days post application compared to Control treatment (Table 4.4b).

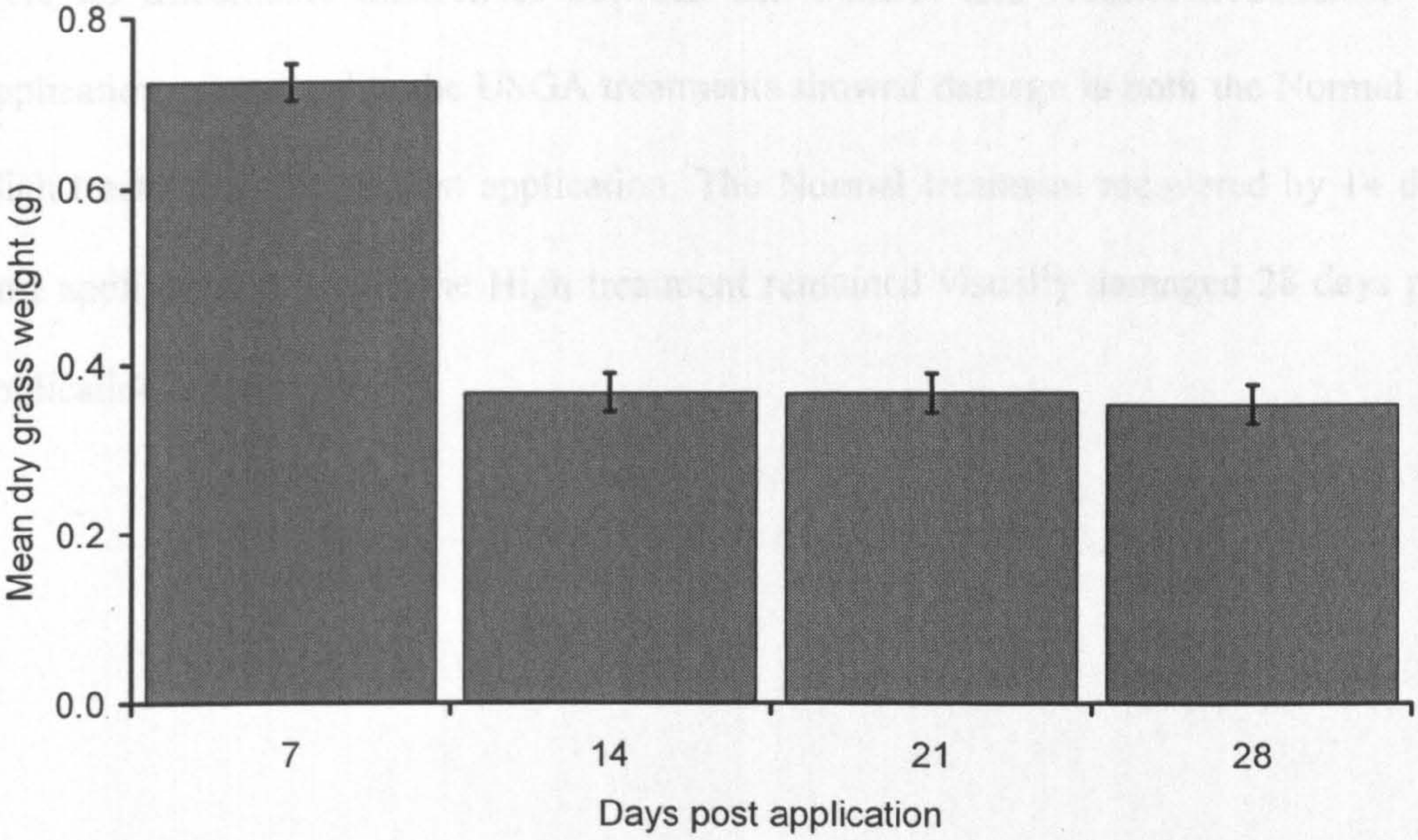


Figure 4.5: Mean dry weight, (n = 7) of grass produced from all treatments. Whiskers show pooled standard error.

Table 4.4: Mean (with standard error) dry weight of grass clippings over 28 days. Tabulated values are g dry weight. Letters indicate heterogeneous groups.

a) Sandy Loam

	Days post application			
Treatment	7	14	21	28
Control	0.99 ± 0.10	0.56 ± 0.03	0.43 ± 0.05	0.38 ± 0.06
Normal	0.87 ± 0.11	0.43 ± 0.06	0.58 ± 0.14	0.45 ± 0.06
High	0.79 ± 0.06	0.44 ± 0.07	0.36 ± 0.03	0.40 ± 0.02
F	1.16	1.74	1.82	0.49
p	0.34	0.21	0.20	0.49

b) USGA rootzone

	Days post application			
Treatment	7	14	21	28
Control	0.54 ± 0.06	0.33 ± 0.05 a	0.45 ± 0.03 a	0.35 ± 0.03 a
Normal	0.68 ± 0.10	0.29 ± 0.03 a	0.27 ± 0.07 b	0.37 ± 0.09 a
High	0.46 ± 0.07	0.15 ± 0.04 b	0.11 ± 0.01 c	0.17 ± 0.02 b
F	1.93	4.84	14.56	3.71
p	0.18	0.02	<0.01	0.05

Visual assessment of the turfgrass showed that while there was no growth effect on the sandy loam treatment there was a change in colour of the turf at the 28 days post application point between the Control and High treatments (Figures 4.6 and 4.7). There

were no discernable differences between the Control and Normal treatments. The application of mustard to the USGA treatments showed damage in both the Normal and High treatments 7 days post application. The Normal treatment recovered by 14 days post application however the High treatment remained visually damaged 28 days post application (Figure 4.7).

Figure 4.6: Photographs of turf grown on sandy loam soil, taken at seven day intervals post treatment application. Individual photographs represent the central point of each pot photographed, scale 1:1.

Figure 4.7: Photographs of turf grown on USGA rootzone, taken at seven day intervals post treatment application. Individual photographs represent the central point of each pot photographed, scale 1:1.

4. 2. 3. Discussion

4.2.3(a) Mustard extraction

The number and species of earthworms found during this survey were consistent with that of other studies in the United Kingdom (Edwards 1996). The proportion of adults to juveniles (40:60) was also consistent with other findings for springtime (Fraser *et al.* 1996).

These experiments demonstrate some major problems with the assessment of the whole earthworm community using mustard extraction. Direct comparison of total numbers extracted and simple species diversity parameters, such as Simpson's diversity index, gave misleading assessment of earthworms present when using a chemical extraction of this type. Work carried out by Lawerence & Bowers (2002) in Virginia, (USA) supports the assertion that mustard extraction is as effective as hand sorting by comparing the mustard expelled population to the residual population. These data agrees with Lawerence & Bowers findings and indicates that we should accept Hypothesis 4.1 (A representative proportion of the extracted earthworm community will be expelled when mustard solution is applied to a defined surface area of turf) that a representative proportion of earthworms are sampled with this method. However, when using a more appropriate control that determines the extant population of earthworms, significant treatment differences in the estimation of the earthworm population are revealed. This implies that mustard extraction may not be as robust a method to assess species diversity of earthworms as has previously been suggested.

However, estimates of species diversity using Simpson's D and E for the extant community and those expelled with mustard extraction show significantly lower

Simpson's D, but equal Simpson's E therefore Hypothesis 4.1 is rejected. For species level data mustard extraction fails to make an accurate estimate of the earthworm population under the quadrat. This could be a Type I error, in that diversity index calculations for both juveniles and adults from each species are considered together. Table 4.1 shows that 60.4% of the earthworms found were juveniles. These juvenile earthworms will have different selective pressures and therefore may be found in different niches in the environment to the adults of the same species.

The community structure shows that populations of total community assessments from each treatment overlap in PC1 and PC 2 (Figure 4.4). These values represent the total community structure in the sample area, and shows they are the same. The standard error associated with the Water (T) treatment is approximately twice that of the Control (T). This indicates that the earthworm community may be affected by the application of water, however 10 L of liquid applied over 0.25 m^2 had no statistically significant effect. Edwards & Lofty (1977) suggest that a mustard extraction followed by hand sorting is the most effective way of estimating earthworm populations. The assessment of the earthworm community structure using mustard extraction and hand sorting would therefore be different to hand sorting alone. This observation is compounded by statistical differences in Simpson's E for these two treatments ($p = 0.04$). These differences indicate that not all earthworms respond in the same way to mustard extraction, broadly supporting the work of Chan & Munro (2001) who report that the anecic earthworms in Australia (*Anisocheatea* sp.) respond to mustard extraction; *Aporrectodea trapezoids* an endogeic species, were unaffected.

The significant difference in PC 1 in species composition between expelled and total fractions (Figure 4.4) means that Hypothesis 4.1 (*A representative proportion of the extracted earthworm community will be expelled when mustard solution is applied to a defined surface area of turf*) must be rejected on the following bases:

1. **Body size.** There is a trend in size of organism, larger loading values from PC 1 correspond to earthworms with a larger surface area, and smaller loading values are represented by earthworms with a lower surface area.
2. **Earthworm maturity.** A trend in maturity down the loading values of PC 1 (see Figure 4.4b), juveniles being negatively loaded and adults positively. This data confirms that the habitat of adult and juvenile earthworms of the same species respond differently to mustard solution. Consequently there may be a Type I error in the analysis of Simpson's D for the same data.
3. **Anecic earthworms.** *Aporrectodea rosea* and *Lumbricus terrestris* are both anecic earthworms which form permanent burrows open to the surface. *Lumbricus rubellus* is an epigeic earthworm with shallow burrows. These three species are represented as the largest loading values on PC1. This indicates a bias towards theses species within the sampling method, further reinforcing the work of Chan & Munro (2001).

Mustard extraction therefore, biases towards large, mainly anecic forms of earthworms.

These results can be explained in terms of the pore structure of the soil. The soil matrix is a complex non-uniform structure, with a range of pore sizes found between the solid constituents (Brady and Weil 1999). Burrowing and subsurface casting of earthworms has been shown to increase the size and distribution of pore spaces (Gorres *et al.* 2001).

The burrows of all earthworms will form macropores, proportional to the body size of the earthworm that created them. The habit of anecic earthworms also means that macropores associated with them will be open at the surface. Smettem (1992) showed that the presence of anecic earthworms producing such macropores has a significant effect on the drainage rate of a soil. This effect has also been recorded in winter sports turf (Baker 1981). The preferential flow of water through the soil means that earthworms in the soil will not all have the same exposure to the mustard solution, especially over the short term. Anecic earthworms in macropores that are large or well connected to the surface will be exposed to the mustard solution first, and hence are more likely to be expelled.

Endogeic and epigeic earthworms will also be exposed to the mustard solution, but over a longer period of time as it percolates thorough the soil through smaller pore networks. Endogeic earthworms may not respond in the same way as anecic earthworms to the mustard solution. The life history of endogeic earthworms is such that they tend to prevail below the surface of the soil. Therefore even if their mucus membranes are exposed to the allyl isothiocyanate from the mustard solution the behavioural response is not necessarily to migrate to the surface and migration of these earthworms horizontally through the soil may be a more likely response. This theory is supported by significant differences in Simpson's E for total earthworm populations. The Mustard treatment shows a significantly lower equitability than both the Control and Water treatments.

4.2.3(b) Phytotoxic effects of mustard solution

Experiments carried out showed characteristic growth trends for both the time of year and the construction material that they were grown on. The weight of dry grass collected per week from the USGA rootzone treatments was significantly lower than the sandy loam treatments at all time points reflecting the lower nutrient and water holding capacity of the USGA rootzone. All treatments including the controls show variability at different time points suggesting that seasonal temperature fluctuations had an impact in this study. Therefore Hypothesis 4.2 must be rejected (*When a mustard solution at standard concentration or at four times the normal concentration, is applied to simulated golf course surfaces there will be no effect to turf health*)

Where the construction material is a sandy loam soil these experiments indicated no phytotoxicity to the turfgrass when using mustard solution. At all time points for both Normal and High concentrations there was no significant difference in dry weight grass yield when compared to the control (Table 4.4a). Visual inspection of sequential photographs however indicates that with the High treatment at 28 days the turfgrass has an increased green hue. This means that no guarantees could be give to green keepers that a mustard solution of any sort will not affect the play surface it is applied to.

4. 3. Implications for earthworm sampling from golf courses

These experiments have major implications to the interpretation of data from mustard extraction surveys. Sampling using this method will be heavily biased towards anecic earthworms and not represent the earthworm community as a whole. In the context of this research, this may not be problematic since it is anecic forms that cast on the surface and so are cause for concern in the golf industry. The growth trials demonstrate that mustard extraction ought not be used on presentation sensitive areas of golf courses

such as tees or greens, since no guarantees could be made that applications will not result in surface damage. Hence such trials need to be confined to fairways.

Chapter 5: Speciation of earthworms on five English golf courses

5. 1. Introduction

Relatively few assessments of earthworm species diversity on golf courses have been carried out. The most recent was conducted in 1997 and was predominantly focused in the north of England (Binns *et al.* 1999). This survey used formalin extraction and was exclusively carried out in the autumn. An assessment of the anecic earthworm population community size and structure were made via mustard extraction, after the seven year systemic effects of the organochloride Chlordane had passed. The findings outlined in Chapter 4.3 limit investigations of earthworm species on golf courses to the least aesthetically important areas of the courses, *viz.* the fairway. The results of earthworm cast surveys (Chapter 3) showed strong seasonal differences in earthworm casting activity. In order to link earthworm species data to these findings a temporal element of investigation is required. The following hypotheses were used to test the causal relationships between these factors:

Hypothesis 5.1. The density and species diversity of earthworms will be different at the surveyed golf courses due to the variations in soil texture at the different sites.

Hypothesis 5.2. The density and species diversity of earthworms will show intra- and inter- annual variations as the organisms respond to changes in environmental conditions.

Hypothesis 5.3. Earthworm species diversity will be positively correlated with the size of the microbial community, governed by an increase in availability of resources that result in a more diverse earthworm community.

Hypothesis 5.4. Earthworm casting activity can be used as an inferred measurement of earthworm species diversity: these two variables will be positively correlated.

5. 2. Materials and methods

Five golf courses (in statistical terms, treatments), as described in Chapter 2.8 were used to test the hypotheses. These treatments are designated:

- John O'Gaunt golf club: Championship course (denoted J)
- John O'Gaunt golf club: Carthagena (denoted C)
- Woburn golf and country club: Dukes course (denoted D)
- Woburn golf and country club: Marquess course (denoted M)
- Buckingham golf club (denoted B)

Mustard extractions, as described in Chapter 4.2 using a 0.25 m^2 quadrat and 10 L of mustard solution (6 g L^{-1}), were carried out in triplicate on the central 100 m^2 of each fairway, at every golf course studied. The location of each quadrat within the 10 x 10 m square was selected at random. Extractions were carried out on three randomly selected fairways per quarter, without replacement (October 2005; January 2006; April 2006; July 2006). This was done at each of the golf courses described in Chapter 2.8 and therefore avoid a nested experimental design (Table 5.1). This design also means that it is not possible to derive statistically valid interaction terms relating courses and sampling time. For investigation of differences *between courses* the replicated experimental unit is the sampling time and for analysis of differences *between sampling times* the replicates are the golf courses.

Prior to the application of the mustard solution, the number of earthworm casts and smears in the quadrat were counted. Earthworms that emerged within 20 minutes after all extraction solution had infiltrated into the soil were collected, washed and stored in distilled water at 4°C prior to speciation. Samples were taken from all golf courses within 10 working days of each other to minimise environmental variation. Two golf courses were excluded from the January sampling time point as the soil was frozen, preventing the mustard solution from infiltrating.

Table 5.1: Fairways sampled (n=5,3 per time point) via mustard extraction through the survey at five different golf courses. Numbers marked with * indicate holes at which no samples could be taken.

	Golf course				
Sampling time	John O'Gaunt	Carthagena	Marquess	Dukes	Buckingham
Oct-2005	3	5	10	15	3
	6	16	16	17	10
	12	3	1	14	14
Jan-2006	11	9	3*	5*	1
	16	10	18*	8*	8
	18	15	4*	12*	11
Apr-2006	5	1	17	18	2
	7	18	2	9	9
	8	12	12	11	13
Jul-2006	1	17	5	1	16
	13	4	14	4	18
	14	8	9	13	17

Five pooled soil cores (25 mm diameter, 250 mm deep) were taken in triplicate from each sampled fairway. This soil was homogenised to pass through a 4 mm sieve at field moisture content and then analysed for microbial biomass carbon (see Chapter 2.6 for methodological details). Other physicochemical parameters used as co-variables in analysis were derived from data used as part of Chapter 3, see Appendix II.

Absolute earthworm populations extracted using mustard solution were calculated per square metre at each time point and for each golf course. Simpson's diversity index (Simpson's D and Simpson's E) were also calculated (see Equations 4.1 and 4.2) based on the species-level data. Rank-abundance analysis was also carried out to determine the most dominant species within the earthworm community. All treatment differences were assessed using one way ANOVA with subsequent post-hoc Tukey HSD for unequal sample sizes. Linear relationships were inferred using general linear models (GLM). Statistics were carried out using Statistica 7.1 (Statsoft Inc. 2005).

5. 3. Results

The range of species recovered from golf course fairways using mustard extraction was consistent with previous findings of this technique in agricultural pastures (Bartlett *et al.* 2006). A total of seven different species were identified, in varying proportions from a total population of 753 individuals. Approximately 2% were unidentifiable juveniles (Appendix III).

5. 3. 1. Between-course variation

The endemic anecic earthworm population of the five golf courses surveyed was found to differ significantly ($p < 0.01$). Earthworm densities were related to their geographical location; both courses at Woburn Golf and Country Club (Marquess and Dukes) supported the smallest earthworm populations. The courses at John O'Gaunt golf club (John O'Gaunt and Carthagena) supported a 4-fold greater population than that found in Woburn. Buckingham supported the largest population of earthworms (Table 5.2). The diversity of these communities is interpreted through Simpson's D and E. The lack of significant differences between any golf course surveyed with regards to Simpson's E indicates that the same kinds of species of earthworms were found at all golf courses,

using the mustard extraction technique. Significant differences in Simpson's D were therefore reflected in the size of the population that the assessment was made on. The most diverse communities (greatest value of Simpson's D) were found where the earthworm populations were highest.

Table 5.2: Mean earthworm density and species diversity indicies relating to different golf courses. Letters indicate homogenous groups determined using Tukey HSD.

Course	Mean earthworm density per m^2	SE	Simpson's D Mean	SE	Simpson's E Mean	SE
Buckingham	34.4 a	3.7	2.00 a	0.26	0.74	0.11
John O'Gaunt	18.9 b	3.7	1.57 a,b	0.25	0.68	0.10
Cartagena	17.6 b	3.7	1.35 a,b	0.25	0.42	0.10
Dukes	5.4 c	4.2	0.93 b	0.29	0.69	0.12
Marquess	2.3 c	4.2	0.88 b	0.29	0.52	0.12
F	8.6		2.8		1.5	
p	<0.01		0.03		0.21	

The size of the earthworm community recovered using mustard extraction can be correlated with soil physicochemical factors. The most significant variable that can be used to describe the earthworm population was the particle size distribution, specifically the sand content (Figure 5.1). This indicates that where there was a high proportion of sand in the soil matrix there were fewer earthworms.

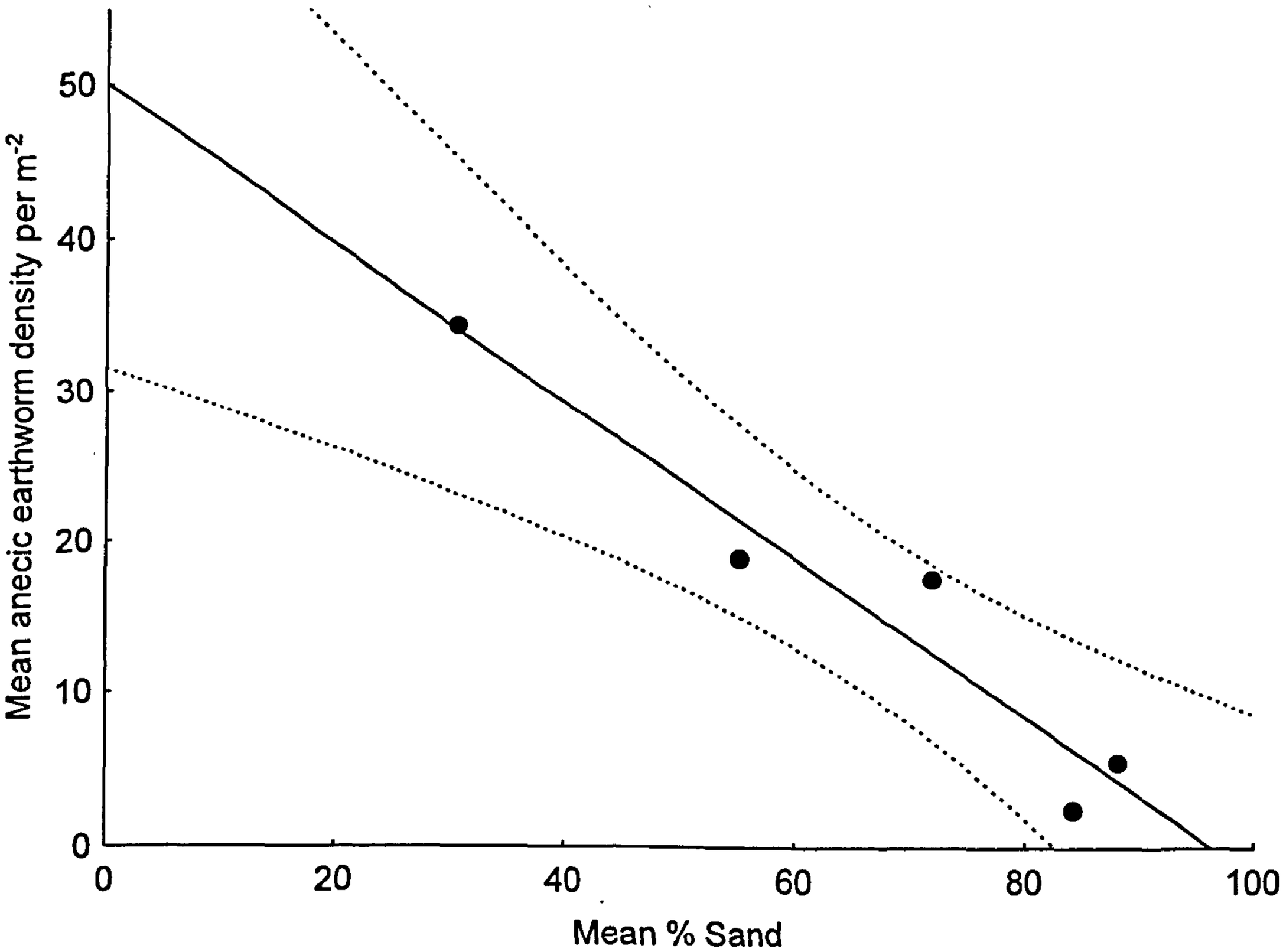


Figure 5.1: Linear increase relationship between mean earthworm density, estimated using mustard extraction and mean sand content of fairway soils at the five golf courses surveyed. Dashed lines show 95% confidence limits; $r^2 = 0.93$; $p < 0.01$.

The size and diversity of the earthworm population was compared to the earthworm surface casting activity, as measured in Chapter 3 ($\Sigma_{cast+smears}$ m^{-2}). There was a poor relationship between $\Sigma_{cast+smears}$ m^{-2} and total earthworm density ($p > 0.05$). However there was a strong relationship between $\Sigma_{cast+smears}$ m^{-2} and Simpson's D; $p < 0.01$ (Figure 5.2). Two significant outliers are obvious, reducing the correlation co-efficient ($r^2 = 0.52$). The equation of this line (see below) is of potential use in estimating the species diversity on other areas of golf courses.

$$\text{Simpson's D} = \frac{\sum_{\text{casts+smears}} \text{m}^{-2} - 0.432}{6.682} \qquad (5.1)$$

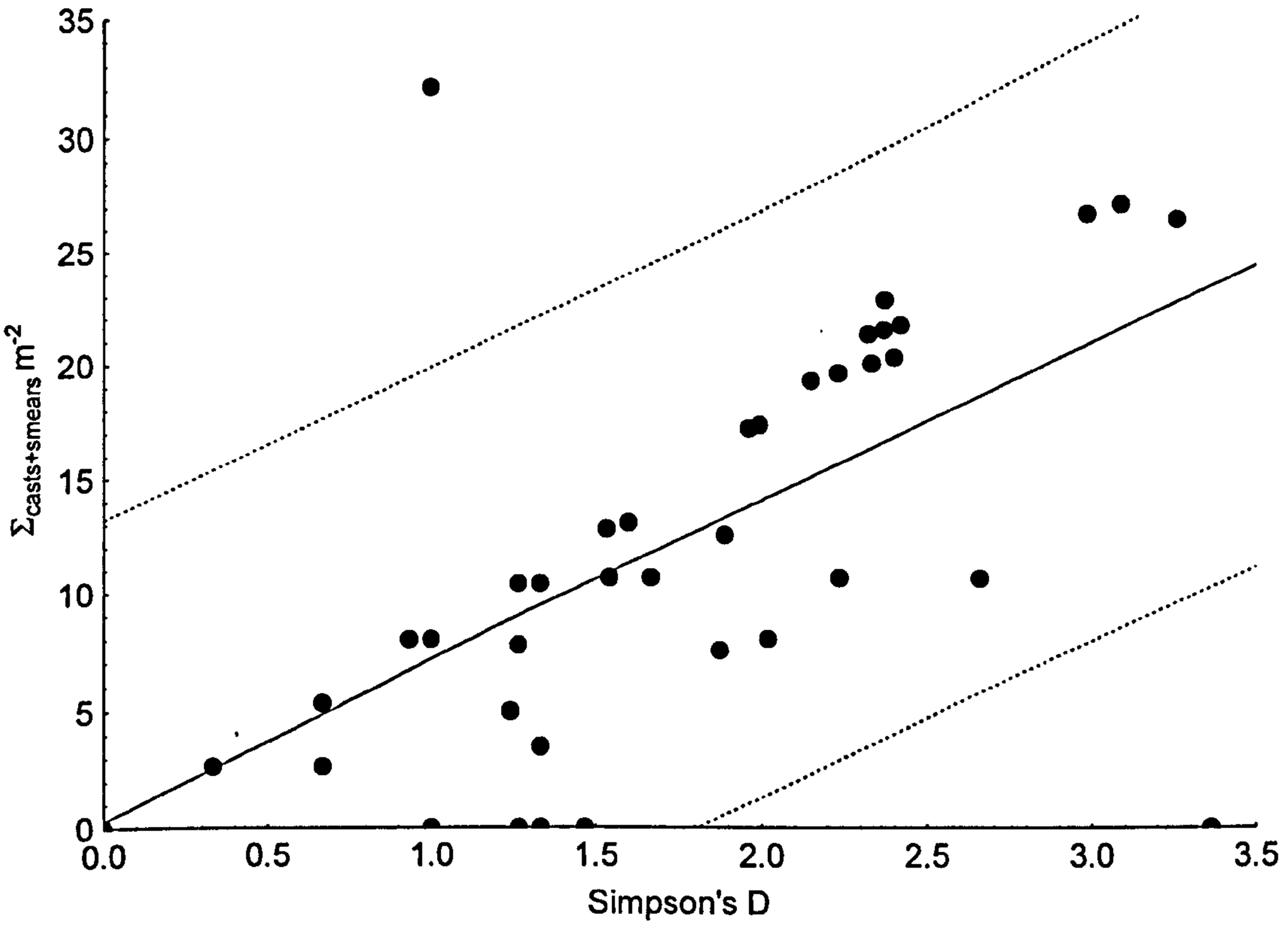


Figure 5.2: The relationship between species diversity index (Simpson's D) and earthworm casting activity ($\Sigma_{cast+smears}$ m^{-2}) at all five golf courses used in the study, at all survey times (n = 54). Dashed lines show 95% prediction interval; $r^2 = 0.52$; $p < 0.01$.

5. 3. 2. Seasonal changes in earthworm populations

There were significant differences between earthworm densities per square metre, and species diversity at different times of year. Principal differences were between summer readings (July) and all other times (Table 5.3). Fewer earthworms, and therefore lower species diversity, was recorded at the summer time point. Absolute anecic earthworm populations estimated using mustard extraction were almost a factor of ten smaller during the summer than at any other point in the year. Similarly estimates of both Simpson's D and E made at other times of the year were approximately twice those

made in July. The trend of low levels of activity during the summer were similar to those recorded in Chapter 3, with regards to earthworm casting activity. Greater variation was recorded regarding Simpson's E; significantly greater evenness was attributed to readings in October than the next two quarters. Measurements of Simpson's E made in July were significantly lower than all other times (Table 5.3).

Table 5.3: Variation in mean (with individual standard error) earthworm density and species diversity indices through time. Letters indicate homogenous groups attributed using Tukey HSD.

Month	Mean earthworm density (number m^{-2})	Mean Simpson's D	Mean Simpson's E	Mean microbial biomass C
Oct-2005	22.3 ± 3.4 a	1.58 ± 0.22 a	0.90 ± 0.07 a	441 ± 88
Jan-2006	28.7 ± 4.4 a	1.85 ± 0.29 a	0.74 ± 0.09 a,b	544 ± 99
Apr-2006	18.8 ± 3.4 a	1.50 ± 0.23 a	0.58 ± 0.08 b	588 ± 144
Jul-2006	3.1 ± 3.4 b	0.80 ± 0.22 b	0.27 ± 0.07 c	447 ± 87
F	8.64	3.46	12.80	0.45
p	< 0.01	0.02	< 0.01	0.71

5. 3. 3. Earthworm species diversity in relation to microbial community size

No significant difference were detected between the size of the microbial community at different times of year that the samples were taken ($p = 0.71$). However microbial biomass C was significantly related to species diversity (Simpson's D); $p < 0.01$. Where microbial biomass C was greater there was a more diverse earthworm community. The correlation coefficient of this line was weak ($r^2 = 0.32$), however, it was unlikely that this relationship was linear due to the complex nature of the interaction of different species present and their competition for resources (earthworm available food in the case of microbial biomass C).

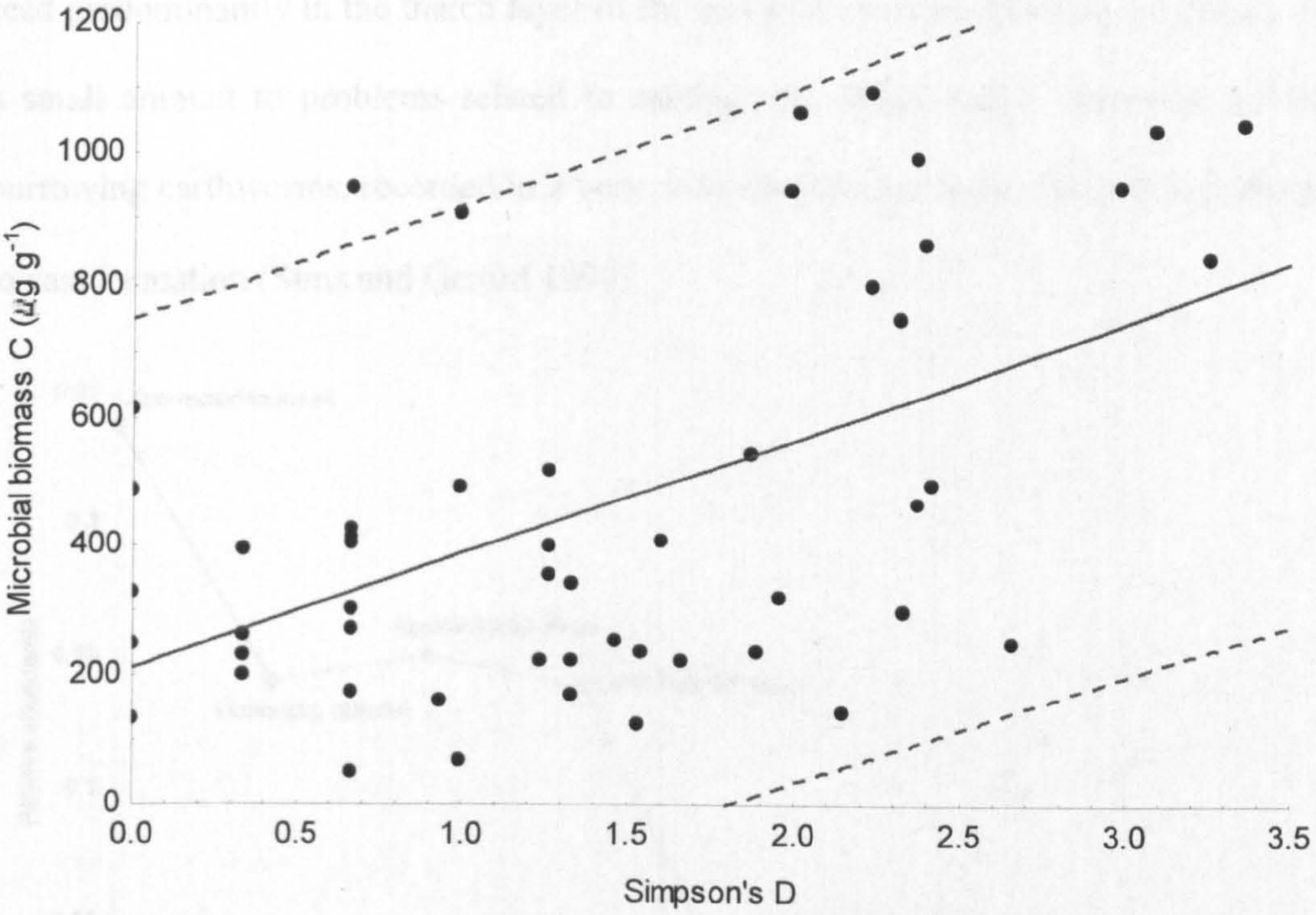


Figure 5.3: The relationship between Simpson's D and microbial biomass carbon (measured by chloroform extraction) adjacent to all mustard extraction plots from all golf courses surveyed, at all time points (n = 54). Dashed lines show 95% prediction intervals, $r^2 = 0.32$; $p < 0.01$.

5. 3. 4. Rank-abundance analysis

Rank-abundance analysis was conceived by Whittaker (1965) and is useful as differences in patterns of species evenness can be highlighted using this approach. When rank-abundance analysis was compared for each golf course, no differences in species evenness were evident (data not shown). Analysis was therefore conducted on the whole data set, increasing the sample size. Figure 5.4 indicates that of the seven different earthworm species *Ap. rosea* was dominant. *L. rubellus*, *Ap. longa* and *L. terrestris* also contribute considerably to the community structure. These four species were a factor of ten more abundant than the others present. The low abundance species were therefore less important with regards to community structure. *Ap. rosea* and *L. rubellus* maintain shallow burrows open to the surface, and are frequently recorded where the soil organic matter is high (Sims and Gerard 1999). These endogeic species

feed predominantly in the thatch layer of the turf grass and are likely to contribute only a small amount to problems related to casting. *Ap. longa* and *L. terrestris* are deep burrowing earthworms, recorded in a very wide distribution and contribute significantly to cast formation (Sims and Gerard 1999).

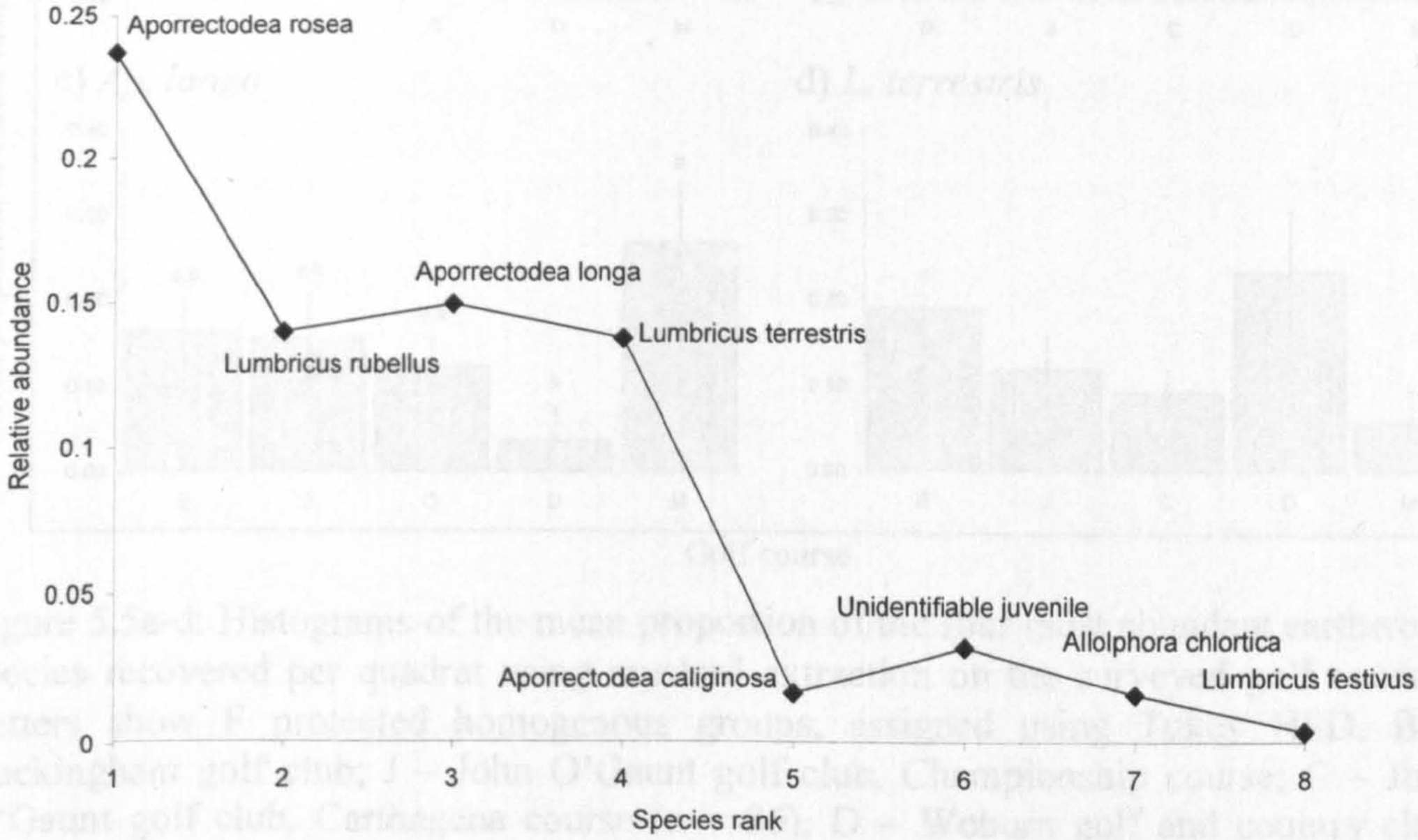


Figure 5.4: Rank-abundance analysis (Whittaker plot) of earthworm species found on all golf courses, at all times of year, n = 162 quadrats per species.

Of the four most abundant species recovered no significant difference was detected between population sizes of *Ap. rosea*, *L. rubellus* and *L. terrestris* between the surveyed courses ($p > 0.05$ in all cases). Significantly fewer *Ap. longa* were recorded at the Dukes course compared to the Marquess course; $p = 0.03$ (Figure 5.5c). A similar (although not statistically significant) trend was seen with *L. terrestris* ($p = 0.06$), where courses at John O'Gaunt and Buckingham golf clubs are not significantly different from each other but fewer of this species were recovered from the other, two courses surveyed. More *L. terrestris* were recorded at the Dukes course in comparison to the Marquess course in Woburn.

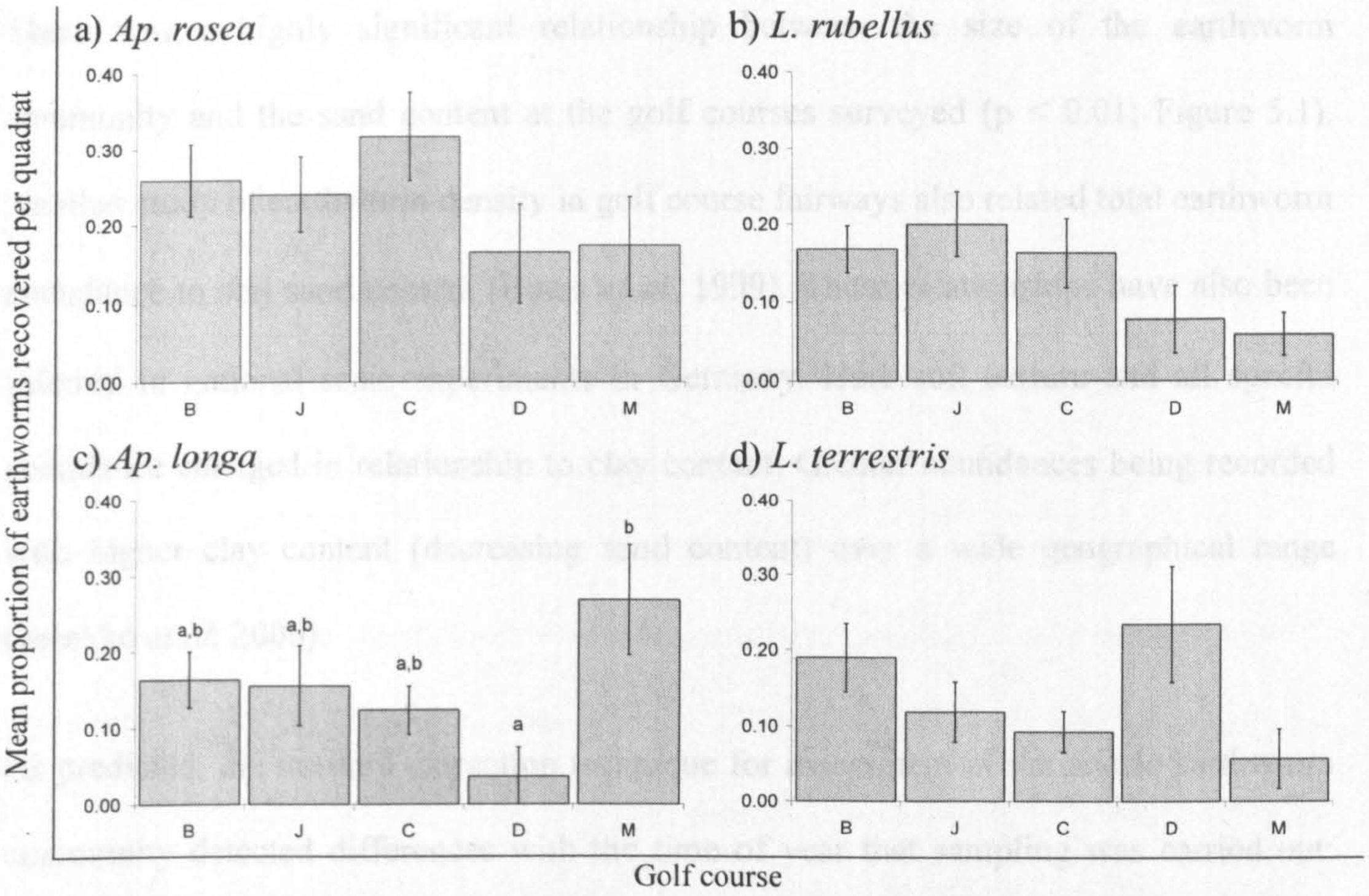


Figure 5.5a-d: Histograms of the mean proportion of the four most abundant earthworm species recovered per quadrat using mustard extraction on the surveyed golf courses. Letters show F protected homogenous groups, assigned using Tukey HSD. B – Buckingham golf club; J – John O'Gaunt golf club, Championship course; C – John O'Gaunt golf club, Carthagena course (n = 36); D – Woburn golf and country club, Dukes course; M – Woburn golf and country club, Marquess course (n = 27).

5. 4. Discussion

It is widely accepted that earthworm community structure ranges widely with different soil type and food source availability, amongst other environmental parameters (Darwin 1883; Edwards and Lofty 1977; Lee 1985). The data recorded over the duration of this experiment indicated that while the (mustard extractable) earthworm community size was variable, the composition of the community was constant at the golf courses surveyed (Table 5.2). These data, in association with soil physicochemical characteristics for the sampled fairways, mean that Hypothesis 5.1 (*The earthworm density and species diversity will be different between the surveyed golf courses due to the variations in soil texture at the different sites*) is accepted.

There was a highly significant relationship between the size of the earthworm community and the sand content at the golf courses surveyed ($p < 0.01$; Figure 5.1). Another study of earthworm density in golf course fairways also related total earthworm abundance to soil sand content (Binns *et al.* 1999). These relationships have also been inferred in national scale experiments in Germany. Here soil texture and all species abundance changed in relationship to clay content. Greater abundances being recorded with higher clay content (decreasing sand content) over a wide geographical range (Joschko *et al.* 2006).

As predicted, the mustard extraction technique for assessment of the anecic earthworm community detected differences with the time of year that sampling was carried out: Hypothesis 5.2 is accepted (*The earthworm density and species diversity will vary through the year as earthworms respond to changes in environmental conditions*). The environmental co-variables used in the temporal model developed in Chapter 3 were not of an appropriate spatial resolution to be compared to these data.

Variations in estimates of both earthworm density per m^2 and Simpson's D followed similar patterns to seasonal variation in $\Sigma_{casts+smears}\, m^{-2}$ on fairways recorded in Chapter 3 (see Figure 3.6c). This suggests that the earthworm community was still active, and relatively close to the surface during the winter (January). The practical details of sampling for earthworms at this time of year were more complex. Where the ground was frozen, a sampling technique that relies on the percolation of water into the soil was unsuccessful. Sampling carried out in July was occasionally problematic in digging the infiltration ring into the ground. Very few earthworms were recovered at this time on

any golf course. Individuals recovered were likely to have been quiescent at shallower depths in order to survive the lower soil moisture conditions.

The widely accepted finding that assessments of both earthworm community size and structure vary with the time of year has most recently been detailed by Jimenez *et al.* (2006). This work showed considerable variation in efficiency of earthworm recovery at different times of the year, with the most inefficient being during the warmest months. They also relate recovery efficiency to earthworm size: smaller worms being less easily recovered. These differences were as a result of the earthworm's biology as a response to the environment. Laboratory experiments have shown relationships with activity and growth rate of *L. terrestris* at different temperatures and soil moistures: less active earthworms being found at higher temperatures and lower soil moistures (Berry and Jordan 2001). These findings complement the perceived wisdom that sampling for earthworms in spring or autumn, when earthworms are at their most active, is optimal (Edwards and Lofty 1977; Lee 1985; Edwards 1996; Doube and Brown 1998).

Investigations carried out in agricultural systems have failed to relate the size of the soil microbial and earthworm communities (Kukkonen *et al.* 2006). These experiments also failed to relate either surface casting activity ($\Sigma_{casts+smears}$ m^{-2}) or the size of the earthworm population with soil microbial biomass ($r^2 = 0.13$ and $r^2 = 0.19$ respectively). Interactions of the soil microbial community with both the soil environment and the earthworm community are complex, with physicochemical parameters having a major impact on the distribution of individuals from within each of these trophic levels. In a long term experiment, where treatments had been in place for over 100 years, relationships between earthworm populations and the microbial community were

evident (Jordan *et al.* 2004). The fairways of the golf courses surveyed in these experiments had a wide range of age of establishment; from five to ninety years. Based on present understanding the potential for treatment differences exists. Detectable differences between areas of a golf course would be unlikely to be between fairways as these surfaces can be considered homogenous with regards to nutrient management. The data did present an interesting relationship between Simpson's D and the size of the microbial community (Figure 5.3). It is unlikely that any relationship between these two variables would be linear, an S-shaped curve is more probable based on these data. (insufficient data to test this hypothesis).

In soils, which are abundant in microbial biomass C, there was a greater availability of resources (food) for the earthworms present, resulting in a wider species range being supported. An increase in resources, results in a more diverse earthworm community, therefore there was a greater turnover of nutrients. By widening the resource parameters the carrying capacity for individual species rises and as a result the population increases towards the carrying capacity. Therefore, a greater proportion of the most adaptive species were present, so more directly occupy the three ecological niches of anecic, epigeic, and endogenic. This reduced niche overlap and so limited both intra- and inter-species competition. These interactions, at a community structure rather than species level, explain both why the relationship exists and why this relationship is more likely to follow an S-shaped curve. A further explanation for this relationship may be the 'hump-back' species resource concept (Begon *et al.* 2005). In this model species diversity follows a parabolic relationship with increased resource availability. The positive correlation coefficient from the linear regression ($r^2 = 0.56$) indicates that if this relation was exhibited here that measurements have only been made on the rising limb

of the parabolic curve or near its apex. Further surveys, carried out in the same way as in this study, at a wider range of golf courses would be required to establish this relationship.

At the community level the ecological groupings of earthworms interact with each other and other components of soil biota in differing ways, but are all related to soil microbes in the same way: the microbes are the primary producers. At the broadest level a relationship with species diversity would be expected (Postma-Blaauw *et al.* 2006). Even within this broad relationship complex interactions have been recorded. Endogeic and anecic earthworms are generally considered to be responsible for reducing microbial biomass, and epigeic earthworms are most frequently implicated in increases in biomass size (Devilegher and Verstraete 1997; Scheu *et al.* 2002). Further research is required to clarify the wide range of interactions between all components of the soil ecosystem and its related food webs.

As stated above, there was no relationship between earthworm casting activity and earthworm population, but a relationship can be inferred between earthworm casting activity and species diversity. Hypothesis 5.4 (*Earthworm casting activity ($\Sigma_{casts+smears}$ m^{-2}) can be used as an inferred measurement of earthworm species diversity: These two variables will be positively correlated*) is accepted. This relationship was a manifestation of the concepts relating to niche occupation by a greater range of earthworm species where resources were more widely available. Where the species richness was greater (increased Simpson's D) there are more earthworms present and the competition for available resources were greater. Measurements of earthworm

communities using mustard extraction is strongly biased to earthworms of anecic habitat (Bartlett *et al.* 2006), therefore the relationship shown in Figure 5.2 describes intra-anecic competition. With regards to optimal patch foraging theory this means that the available resources per earthworm are lower in a more diverse community and therefore each individual must move to a new patch with increased frequency (Charnov 1976). Where species richness was high and evenness was homogenous, the resulting large population means that each conceptual feeding patch was relatively small. Earthworms generally feed within a short distance from their burrow, inside an estimated 450 mm radius (Nuutinen and Butt 2005). When moving to a new feeding patch they must therefore make a new burrow, resulting in the formation of more casts.

5. 5. Species relating to earthworm problems on English inland golf courses

In order to direct appropriate control mechanisms for earthworms it is important to know what the most dominant species of earthworms found on golf courses are, and how they interact with the environment. Rank-abundance analysis showed that two species of shallow burrowing earthworms were most dominant (*Ap. rosea* and *L. rubellus*) and two deep burrowing earthworms were present in significant numbers (*Ap. longa* and *L. terrestris*); see Figure 5.4. All of these earthworm species maintain permanent or semi permanent burrows that were open to the soil surface (Sims and Gerard 1999) and as such are potential candidates to cause problematic earthworm casts on golf courses.

Aporrectodea rosea is a relatively small earthworm generally between 25 – 85 mm long and between 2 – 6 mm in diameter and has been recorded in a wide range of soils both nationally and internationally, it has a high recorded abundance in pastures and will

survive soil pH between 4.9 and 9.8 (Sims and Gerard 1999). *Ap. rosea* has been shown to have a high fecundity in comparison to many other earthworm species (Holmstrup 1999). In benign soil conditions, as present in the fairway of a golf course, rates of reproduction are likely to be high. A species with this kind of reproductive biology will dominate in soils that had previously been denuded of earthworms through the use of vermicides with long systemic effects such as Chlordane. *Ap. rosea* is not likely to create significant amounts of surface casts and so is of relatively little importance to green-keepers with respect to the control of earthworm casts on golf courses.

Lumbricus rubellus is a considerably larger earthworm, adults are between 60 – 130 mm long and between 3 – 4 mm in diameter and are again recorded in a wide range of soil pH (3.5 – 8.4). Their distribution is consequently widespread but are predominantly found in moist soils high in organic matter (Sims and Gerard 1999). *L. rubellus* has been observed as a primary detritivore, feeding on organic debris both on the soil surface and in the matrix (Seeber *et al.* 2006). On golf courses where the turf is cut regularly there is a constant supply of both of these sources of food. Regular above ground biomass removal encourages the turf grass plant to increase the root growth (thatch formation). Mowing causes damage to the shoot apical meristems, one consequence of this is the increase of the amount of plant growth regulators produced by the plant, such as indole-3-acetic acid (IAA). The action of IAA on all apical meristems (both root and shoot) is to cause cell elongation and division, hence stimulating root growth. Where mowing occurs frequently, and at an appropriate height the plant stimulation causes root growth, and thatch formation (Emmond 2000; Sailsbury and Ross 1992). Consequentially there is a plentiful supply of food preferable

to this species in the soils of golf courses. These two dominant zones for feeding means that the burrow structures of *L. rubellus* must be open to the surface. This species of earthworms also has a high environmental resilience, being tolerant to a wide range of toxic soil conditions. Its reproductive plasticity means that it is very hard to exterminate (Klok *et al.* 2006). These features of its biology mean that *L. rubellus* is frequently studied as an indicator of soil heavy metal contamination (Brown *et al.* 2004; Vijver *et al.* 2005; Hobbelen *et al.* 2006). Such a resilient species is unlikely to have been significantly impacted for any great length of time with previous chemical controls that have been used at golf courses. This is reflected in the high relative abundance recorded.

Aporrectodea longa is another large earthworm with adults being between 90 – 170 mm and between 4 – 9 mm in diameter. They are widespread in the temperate regions of the Northern hemisphere and have been introduced into temperate zones of Southern hemisphere. *Ap. longa* are common in pastures and cultivated soils, with an optimal soil pH range between 6.7 – 9.4 (Sims and Gerard 1999). Where this species of earthworm have been introduced in Australia they have been demonstrated to be formidable competitors for resources (Baker *et al.* 2002). The wide range of soil types that they are adapted to living in gives them this significant advantage. They are however, not successful in soil temperatures greater than 15°C and related lower soil moisture contents, although *Ap. longa* cocoons are capable of surviving in relatively dry soils (≈ 6 – 8% soil moisture). Hence these earthworms form deep, large burrows, in order to remain within optimal environmental parameters (Baker and Whitby 2003). Large, deep and permanently maintained burrows mean that the earthworm must evacuate large

volumes of soil, therefore forming copious numbers of large casts. This species is likely to present one of the greatest problems to green keepers.

Lumbricus terrestris is one of the largest species of earthworm found in UK soils. Specimens have been recorded up to 350 mm but adults are generally between 90 – 250 mm with a diameter of 6 – 10 mm. Earthworms of this size have correspondingly large burrows, some of which have been recorded to depths of up to 3 m (Lee 1985). They are most frequently recorded in undisturbed soils with relatively high pH, when compared to the tolerance of other earthworm species: between pH 6.2 – 10.0 (Sims and Gerard 1999). In earthworm surveys of arable fields *L. terrestris* have been found in greatest abundances in proximity to where land drains have been installed, indicating a preference for freely drained soils (Nuutinen *et al.* 2001).

The engineering of all of the fairways surveyed in this study had some form of drainage either as permanent land drains, mole drains or sand slit drains to insure that fairways do not become waterlogged during periods of heavy rainfall (*golf course managers; personal communications*). This facet of golf course construction may be providing an ideal habitat for this species of earthworm that will be responsible for considerable amounts of surface casting based on its size alone. The most comprehensive survey of earthworm species conducted prior to this, carried out in 1997 (Binns *et al.* 1999) indicates that *L. terrestris* is the most abundant species of earthworm on golf courses. *L. terrestris* was only the fourth most dominant species on these golf courses. Disparities in these findings could be due to the fact that since highly toxic organochlorides, like Chlordane have been banned under the Water Framework Directive the gross earthworm community structure has changed. Equally, differences in sampling

technique and survey structure, time of year and geographical location may have caused the differences in observation. Irrespective of the order of the abundance of this earthworm species (recovered in 15% of all quadrats; Figure 5.4) its size and feeding habits, both surface and within soil matrix foraging, mean that it presents a significant problem to green keepers in minimising surface casts from earthworms.

These surveys have identified the potential earthworm pest species, however, no confirmations can be made that any of these species are responsible for casting on the surface of golf courses as no earthworms were ever observed making casts. The problem of species identification of which earthworms are responsible for the cast formation could be removed by direct cellular DNA analysis from the soil of the casts. When earthworms ingest soil and pass it through there guts the abrasive nature of the soil particles will cause some of the gut wall lining to be sloughed and excreted with the rest of the soil as a cast. Harper *et al.* (2005) have shown that DNA fragments can be recovered from the guts of invertebrate predators. Using a multiplex PCR technique they were able to recover DNA from earthworms in post digestion gut contents 96 hours after excretion. The conditions within the earthworm gut are likely to be more benign with regards to earthworm cell degradation than that of the predators which they studied. Therefore these findings suggest that identifiable earthworm casts found on golf courses (which have not been smeared by mowing) are likely to contain amplifiable earthworm DNA. There are several problems with this methodology which require further research (and were beyond the scope of this thesis). Harper *et al.* (2005) also report that while they could amplify and identify earthworm DNA they were unable to make species distinctions based on these results. Further research, expanding the gene library for

earthworm DNA would be required to make a more accurate, species level assessment of the earthworm that had produced the cast. By expanding this research direct identification of earthworms would be possible from the casts formed on the surface.

The most comprehensive survey of earthworm species conducted prior to this, carried out in 1997 (Binns et al. 1999) indicates that *L. terrestris* is the most abundant species of earthworm on golf courses. *L. terrestris* was only the 4th most dominant species on the golf courses surveyed in the present study. Disparities in these findings could be due to the fact that since the prohibition of use of organochlorides, the gross earthworm community structure has changed. Equally, differences in sampling technique, survey structure, time of year and geographical location may have caused the differences in observation. To golf course managers it is only the surface active, anecic earthworms that present any significant management problems. This study indicates that the abundance and community structure of these species may now be significantly different compared to historic surveys or green keepers' personal perceptions. The identification of the most dominant surface extractable earthworm (*Ap. rosea*, *L. rubellus*, *Ap. longa* and *L. terrestris*) means that research into environmentally benign controls for earthworm casting on amenity turf can be better directed in the post-Chlordane chemical use era.

Chapter 6: Inter-course microbial analysis: The interactions of microbial community structure and the soil environment of golf courses

6. 1. Introduction

There are little comprehensive data available about the microbial community structure found in the soil of any golf course surfaces. Work that has been carried out has been principally by agrochemical companies to produce products that 'promote' the activity of bacteria and fungi and as such are marketed as soil conditioners to improve turf grass growth (Mueller and Kusow 2005). Some assessments have been made by the USGA, however, these have predominantly been measured *in vitro* using culture enrichment colony counting techniques (Karp and Nelson 2004). This method has been shown to be un-representative of community structure isolating less than 1% of the extant community (Brock 1987; Saleh-Lakha *et al.* 2005). The soil microbial community is a major contributor to nutrient cycling and food webs within the soil. It is of critical importance to earthworms who are incapable of producing the cellulase and hemicellulase enzymes required to break plant tissue down. There is evidence linking the quantity of soil microbial biomass with the presence and activity of earthworms (Doube and Brown 1998; Lavelle 2001; Gorres *et al.* 2001). These findings would imply that in areas of a golf course where there is increased microbial activity, from a more diverse microbial community, there will be increased earthworm activity.

Soil biota genotype varies over different soil types and geographical locations (Tiedje *et al.* 1999) however, it is their interaction with the environment that governs the expression of the microbe's genotype as its phenotype. By assessing the microbial phenotype expressed in the soil, and therefore the microbial community structure,

relationships about the cycles and processes that are active within the soil can be inferred. Land management techniques have often been shown to alter the soil microbial community structure (Zelles 1999). Work carried out in arable systems has shown that crops that receive high dosages of fungicidal chemicals have an altered microbial community structure. All golf course surfaces studied here are sprayed with fungicides and have fertilisers applied, but at differing rates and frequency. These differences are likely to be reflected in the microbial community found within the soil. It is hypothesised that the level of maintenance that different areas of the golf course are subjected to affects the expression of the phenotypic community structure of soil microbes due to the construction and maintenance of each different golf course. Each play surface on the golf courses surveyed was recorded to be distinctly different physical environment (see Figure 3.4). These environmental factors controlling gene expression and microbial survival will impact the microbial community structure in a different way on each of the golf courses surfaces.

To investigate the differences in microbial community structure on golf courses the following hypotheses were tested:

Hypothesis 6.1. Different construction materials and maintenance regimes will result in a different phenotypic expression from the soil microbial community due to its response to treatment (surface) specific environmental parameters.

Hypothesis 6.2. Geographically dispersed golf courses will have different soil microbial community structures due to variation in soil chemistry and other environmental parameters.

6. 2. Materials and methods

Samples were taken in early March 2005 from the five golf courses in Bedfordshire and Buckinghamshire, described in Chapter 2.8.

6. 2. 1. Experimental design

The six surface types found on golf courses were identified and used as (statistical) treatments. These surface types are each similar in construction and maintenance procedures irrespective of their geographical location:

- Standard tee (designated T)
- USGA topped tee (designated UT)
- Fairway (designated F)
- Standard green (designated G)
- Temporary green (designated TG)
- USGA green (designated UG)

Sub-categorisation was made on Buckingham Golf Course where USGA greens and temporary greens where present, similarly tees constructed with a 100 mm band of USGA rootzone were only present on the Marquess course, Woburn Golf and Country Club.

At each golf course five tees, fairways and greens were randomly selected to be sampled (Table 6.1). These surfaces were sampled in a similar way to the quadrat surveys. Soil cores were taken on each surface from the nodes of a W-of- best fit on tees and greens (n=5, Figure 6.1) and were pooled in the field within each surface replicate. On the fairways, a stratified random design was used, ten evenly sized strata were imposed on the length of the fairway and one soil core was taken from a randomly

selected position within each stratum (n=10, Figure 6.1). These samples were also pooled in the field.

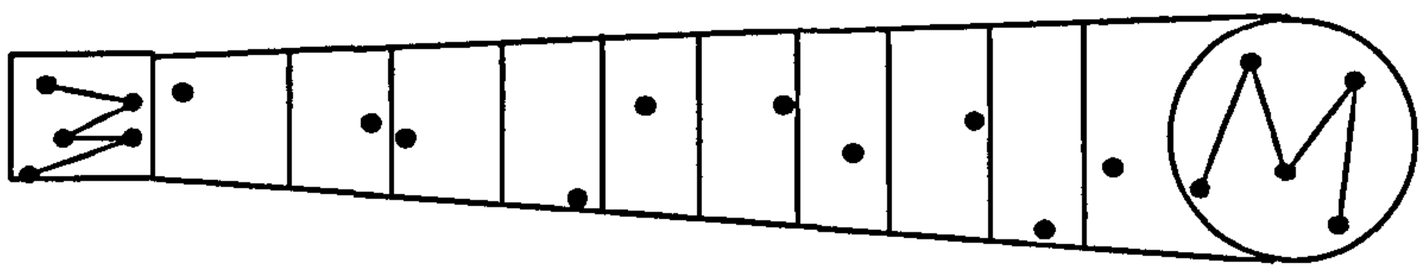


Figure 6.1: Stylised distribution samples taken from tees, fairways and greens

Each soil core was taken with an auger (10 mm diameter) to a depth of 250 mm. To limit root material contamination of the samples the top 25 mm of each soil core was discarded. Soil samples were stored at 4°C for seven days prior to sieving at field moisture content to pass a 4 mm sieve. Samples were then frozen at -86°C and freeze-dried using an Alpha 1-2 LD freeze dryer (Christ Freeze Driers, Osterode-an-Harz, Germany).

6. 2. 2. Laboratory analyses

An assessment of the phenotypic structure of the microbial community was assessed using phospholipid fatty acid analysis (see Chapter 2.8). The relative concentration of each PLFA was expressed on a mol % basis – see Appendix IV.

Table 6.1: Holes (randomly selected) from which soil samples were taken at each different play surface of the golf course[7].

	Standard tee	USGA topped tee	Fairway	Temporary green	Standard green	USGA green
John O'Gaunt	1	-	10	-	6	-
	17	-	9	-	13	-
	8	-	1	-	4	-
	9	-	14	-	17	-
	5	-	16	-	16	-
Carthagena	12	-	4	-	5	-
	6	-	8	-	1	-
	2	-	1	-	14	-
	13	-	3	-	11	-
	4	-	18	-	13	-
Buckingham	17	-	14	17	-	12
	6	-	1	6	-	5
	12	-	3	12	-	17
	3	-	17	3	-	14
	5	-	14	5	-	13
Marquess	-	16	6	-	-	17
	-	8	1	-	-	5
	-	17	14	-	-	15
	-	5	8	-	-	18
	-	6	9	-	-	1
Dukes	6	-	6	-	12	-
	3	-	1	-	5	-
	8	-	7	-	17	-
	18	-	17	-	14	-
	7	-	13	-	13	-

6. 2. 3. Data analysis

Principal components analysis (PCA) using covariance and *post-hoc* one way analysis of variance (ANOVA) was carried out on mol % data to determine ecologically relevant structures within the data. Canonical correlation analysis (CCA) was used to determine correlations between the physical environment on the phenotypic community structure. The following physical factors measured for each surface (derived from the co-variables used in Chapter 3 – Appendix II) were used for this statistic:

- Sand (%)

[7] Pictographs of spatial orientations of golf courses can be found in Chapter 2.8.

- Clay (%)
- Loss on ignition (%)
- Cation exchange capacity (CEC)
- pH
- Total organic carbon (% TOC)
- Total nitrogen (% N)
- Total carbon (% TC)
- C:N ratio

All analysis was carried out using Statistica 7.1 (Statsoft Inc, 2005)

6. 3. Results

6. 3. 1. Principal components analysis

In the analysis of phenotypic community structure using PCA for all data, from all surfaces and golf courses, 94% of the variation was accounted for by the first five components (PC). ANOVA for each of these PCs showed significant surface treatment differences between samples taken on all golf courses in only PC1 ($p < 0.01$) and PC5 ($p < 0.01$). Significant differences in phenotypic community were also recorded between golf courses measured on PC1 ($p < 0.01$) and PC4 ($p = 0.03$); Table 6.2.

Graphical analysis of the significant components with respect to surfaces (Figure 6.2a) showed three distinct groupings of data with surfaces that include USGA rootzone in their construction being negatively loaded on PC1, and the temporary greens being positively loaded on the same axis. Fairways and standard tees and greens were neutrally loaded in both axes. Negative weightings in these groups indicated communities dominated by Gram-negative bacteria (16:1ω7 c and 16:1ω7 t) and a range

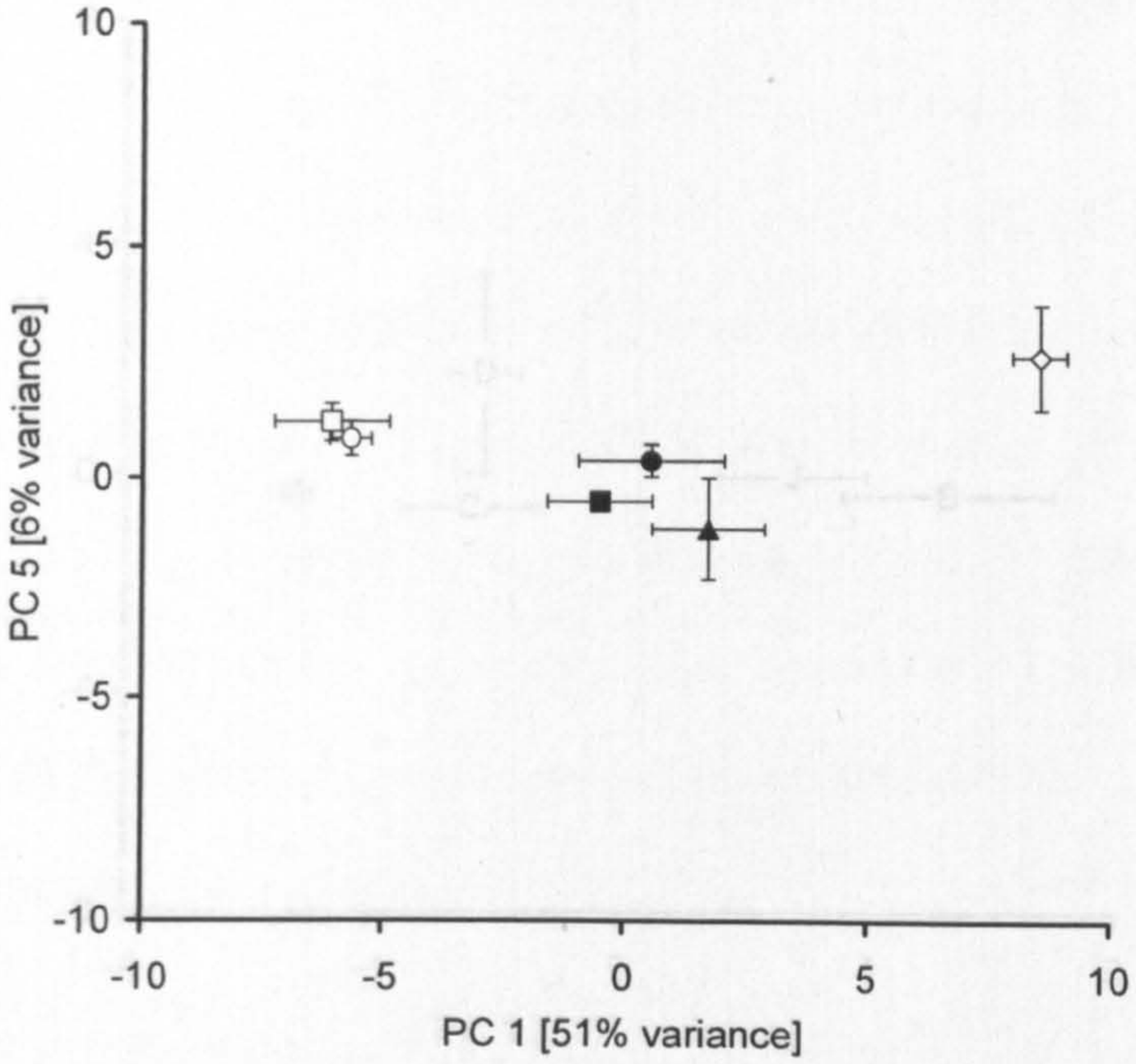
of potential communities, but most ecologically relevant, Type I methanotrophs (16:0). PLFA biomarkers found to be prevalent in communities dominant by Gram negative bacteria (18:1ω9 t and 19:0 c) were positively weighted (Figure 6.2b).

A projection of PC1 and PC4 grouped by golf courses showed three clusters on PC1, Marquess; Dukes and Carthagena; John O'Gaunt and Buckingham, from negative to positive loadings respectively (Figure 6.3a). These groupings were not an artefact of the relationships shown in Figure 6.2a as there were no similarities in any orientation between different surface type and golf courses. The weightings attributed to these delineations of community structure are dominated by Gram-negative bacteria and Type I methanotrophs (16:1ω7 c, 16:1ω7 t and 16:0 respectively) in the negative weightings and Gram-positive bacteria (18:1ω9 t, 18:1ω9 c and 19:0 c) in positive weightings (Figure 6.3b).

Table 6.2: One-way ANOVA of PC 1-5 grouped by surface (n=6) and by course (n=5).

		Grouping variable			
		Surface		Course	
	% variance	F	p	F	p
PC 1	51.2	7.08	< 0.01	13.52	< 0.01
PC 2	12.4	1.13	0.35	1.98	0.11
PC 3	8.0	2.09	0.08	1.37	0.25
PC 4	7.4	0.66	0.66	2.85	0.03
PC 5	6.2	4.89	< 0.01	13.14	< 0.01

a)



b)

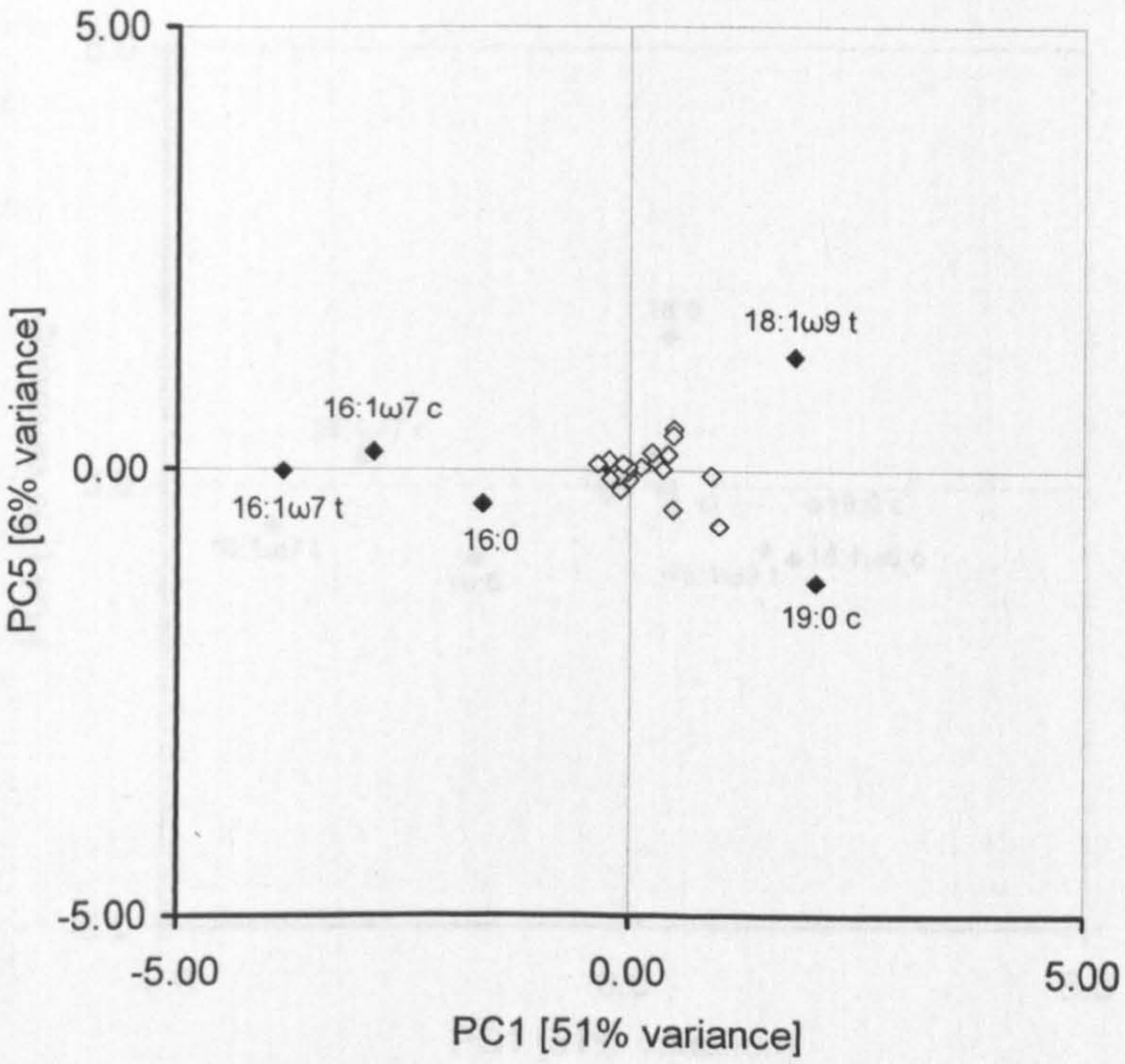
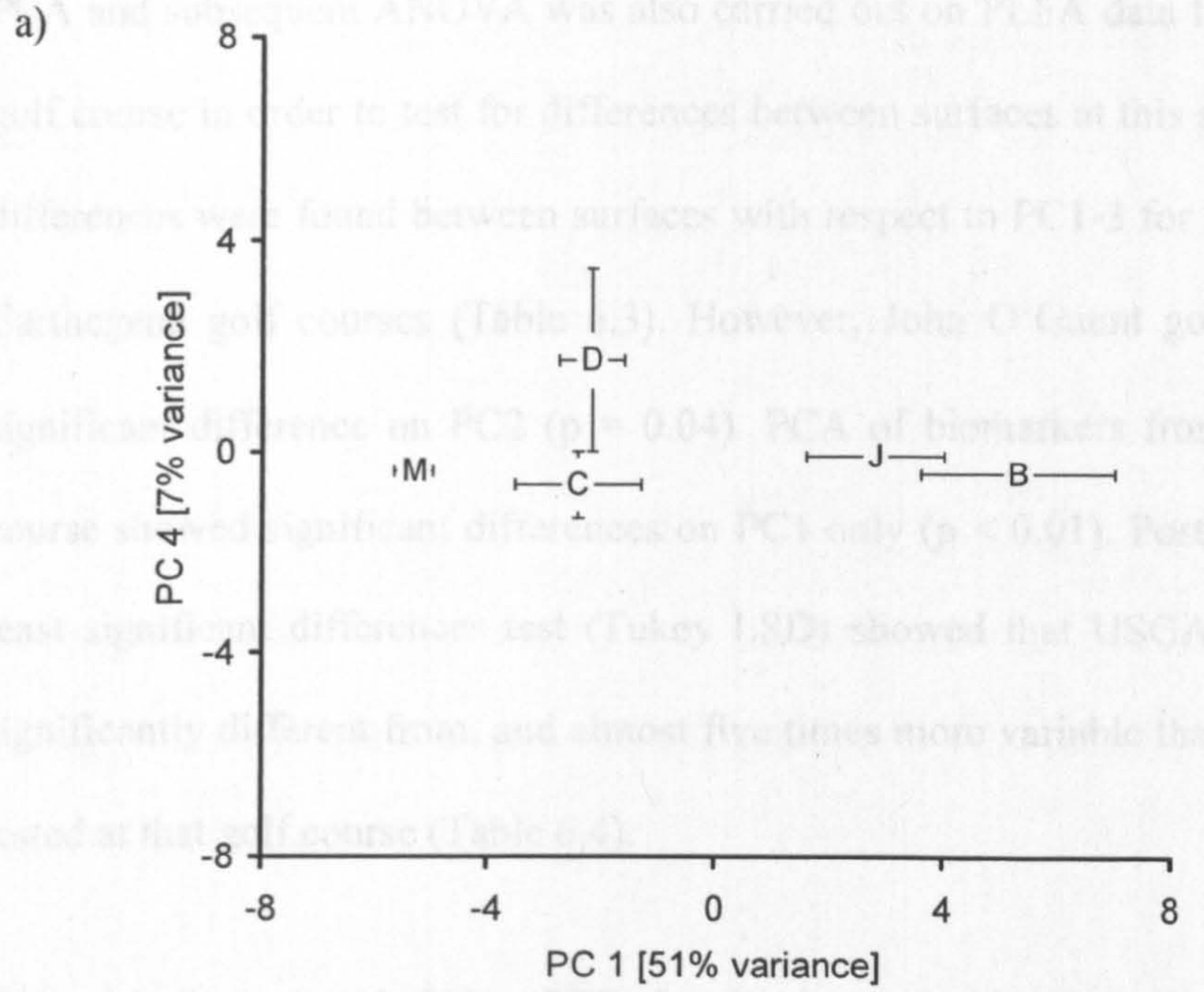


Figure 6.2: Principal Component (PC) analysis of PLFA data derived from five golf courses. a) Projection of mean co-ordinates of significant PCs with respect to tees (●), USGA topped tees (○), fairways (▲), greens (■), USGA greens (□) and temporary greens (◊) found on golf courses. Whiskers show standard error of the mean. b) Dominantly weighted loading values of PLFAs attributed to significant differences between surfaces projected in Figure 6.2a, most significant variables denoted with closed symbols.



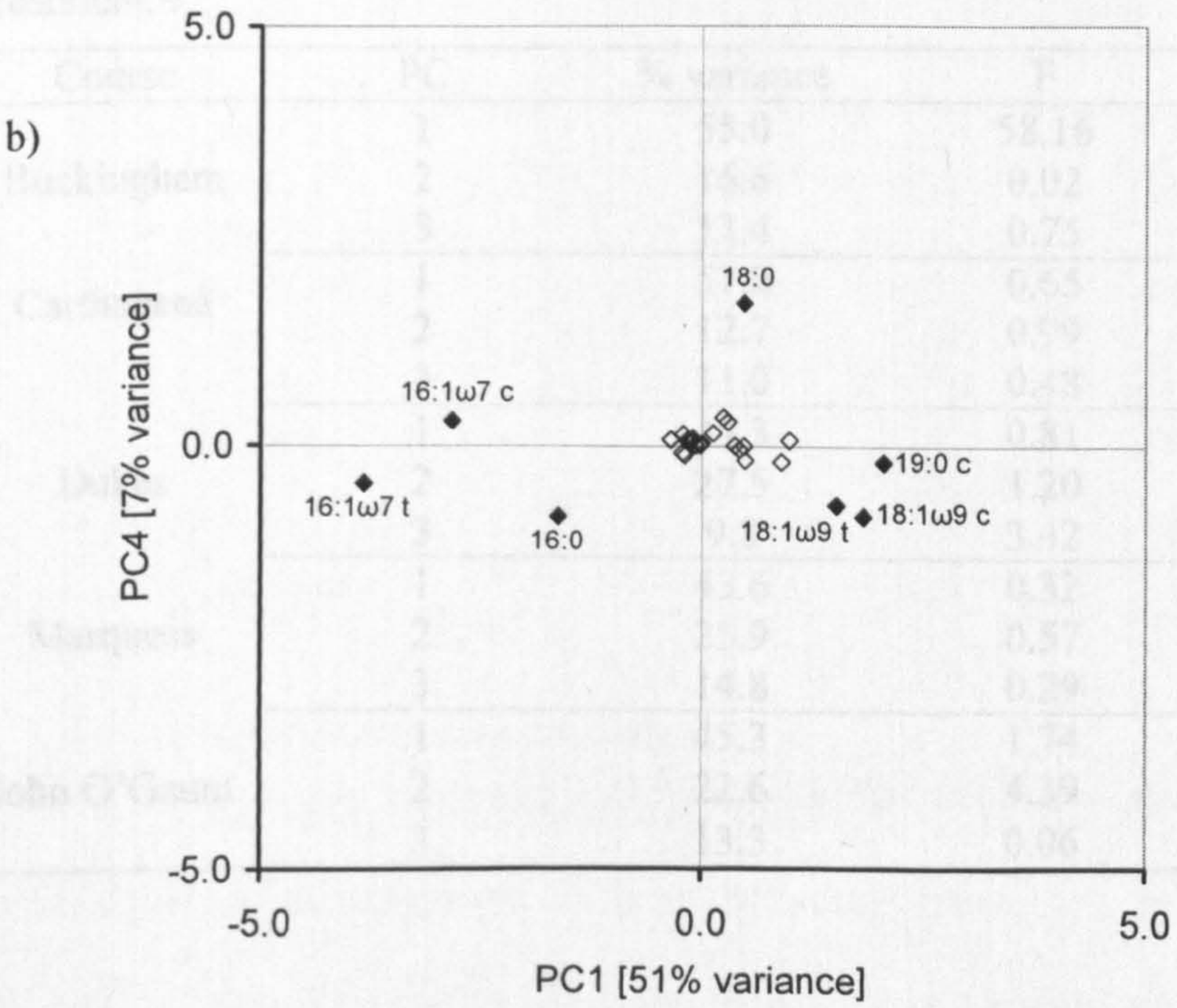


Figure 6.3: Principal Component (PC) analysis of PLFA data derived from five golf courses. a) Projection of mean co-ordinates of significant PCs with respect to Marquess (M); Dukes (D); John O'Gaunt (J); Carthagena (C); Buckingham (B) golf courses. Whiskers show standard error of the mean. b) Dominantly weighted loading values attributed to significant differences between surfaces projected in Figure 6.3a closed symbols indicate the most significant variables.

PCA and subsequent ANOVA was also carried out on PLFA data from each individual golf course in order to test for differences between surfaces at this scale. No significant differences were found between surfaces with respect to PC1-3 for Marquess, Dukes or Carthegena golf courses (Table 6.3). However, John O'Gaunt golf course showed a significant difference on PC2 ($p = 0.04$). PCA of biomarkers from Buckingham golf course showed significant differences on PC1 only ($p < 0.01$). Post-hoc analysis, using least significant differences test (Tukey LSD) showed that USGA greens (UG) were significantly different from, and almost five times more variable than, all other surfaces tested at that golf course (Table 6.4).

Table 6.3: One-way ANOVA of PCs 1-3 from each individual golf course, n=5 per treatment.

Course	PC	% variance	F	p
Buckingham	1	55.0	58.16	< 0.01
	2	16.6	0.02	> 0.99
	3	13.4	0.75	0.54
Carthagena	1	57.2	0.65	0.54
	2	12.7	0.99	0.40
	3	11.0	0.48	0.63
Dukes	1	44.3	0.81	0.47
	2	27.5	1.20	0.34
	3	9.8	3.42	0.07
Marquess	1	43.6	0.32	0.74
	2	25.9	0.57	0.58
	3	14.8	0.29	0.75
John O'Gaunt	1	45.3	1.74	0.22
	2	22.6	4.39	0.04
	3	13.3	0.06	0.94

Table 6.4: Breakdown of differences between fairways (F), standard tees (T), temporary greens (TG) and USGA greens (UG) within PC1 from Buckingham golf club. Values not followed by same letter significantly different using least significant differences test. n=5 per treatment.

Surface	Mean PC 1	S.E.
F	5.13 a	0.35
T	4.03 a	0.38
TG	3.40 a	0.58
UG	-12.56 b	2.06

Linear relationships between PC1 and physical factors from each surface measured with a general linear model showed a poor fit between physical factors and the PLFA data ($p > 0.05$ in all cases; Table 6.5).

Table 6.5: Probabilities of relationships (p) between physical factors and PC1 from analysis of all data points (Figure 6.2) using a general linear model.

Physicochemical attribute	F	p
pH	1.55	0.22
CEC	0.14	0.71
Loss on ignition (%)	0.12	0.73
Sand (%)	2.97	0.09
Clay (%)	2.70	0.11
Silt (%)	2.50	0.12
N (%)	0.42	0.52
TC (%)	0.03	0.86
C : N Ratio	0.45	0.51
TOC (%)	2.28	0.14

6. 3. 2. Canonical correlation analysis

CCA is a regression technique which allows for the analysis of two independent multivariate sets of data. In the analysis, both multivariate data sets are independently resolved in multi-dimensional space and then each axis is mathematically transformed to have a mean of zero and standard deviation of one: this is defined as the canonical root. These transformed co-ordinates and variable weightings allows for interpretation of significant relationships between the sets of data by regression of both the Canonical

Root 1 (CR1) data from each multivariate data set (ter Braak 1996; ter Braak and Smilauer 2002). When both physical and PLFA variables from all golf courses were subjected to this analysis a highly significant relationship was inferred ($p < 0.001$, canonical $R = 0.96$). A projection of $CR1_{(Physical)}$ vs. $CR1_{(PLFA)}$ demonstrates this (Figure 6.4a). Grouping of variables along a straight line intimates significant relationships. Significant differences were recorded on both $CR1_{(Physical)}$ and $CR1_{(PLFA)}$; $p < 0.01$ in both cases. Three distinct clusters were identified using Tukey honest significant difference: USGA greens and USGA topped tees; fairways and standard tees; temporary greens. Standard greens were intermediate to both USGA topped tees and standard tees. The distribution of these groups was interpreted by the weightings for CR1 in the orientation in which they affect the data (Figure 6.4b) and the amount of variance associated with each variable in the order-of-magnitude of this effect (Table 6.6).

The values of physical weights were interpreted as most important because $CR1_{(Physical)}$ accounts for almost twice the variance of $CR1_{(PLFA)}$, hence sand (%) has a strong negative effect and % N has a strong positive effect on the orientation of variables in Figure 6.4a. From the nine variables that account for more than 50% of the variance associated with CR1, two-thirds were physical factors. The most significant PLFA biomarkers were 16:1ω7 c, an indicator of communities dominated by Gram-negative bacteria and 17:0 isomers, a eubacterial biomarker.

This analysis resolves clear physicobiological differences between golf courses (Figure 6.6). Each golf course forms a discrete group on both axes ($p < 0.01$). These groups

reflect spatial relationships between the golf courses, with golf courses in similar geographical locations being adjacent in this projection.

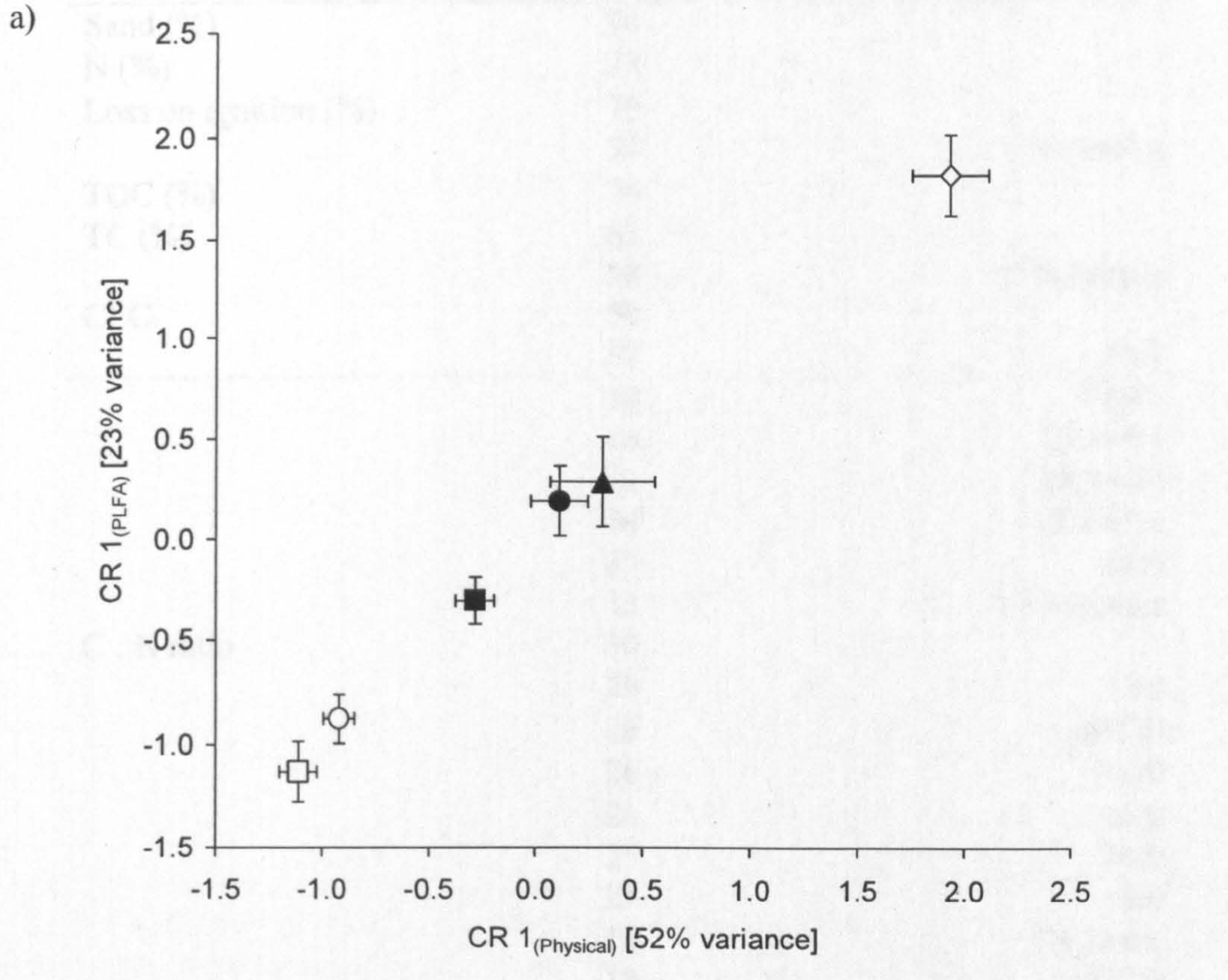


Figure 6.4: Canonical correlation analysis of PLFA data and physical soil characteristics derived from five golf courses. a) Projection of mean co-ordinates of each canonical root (CR) with respect to standard tee (●), USGA topped tee (○), fairways (▲), standard greens (■), USGA greens (□) and temporary greens (◊) found on golf courses. Whiskers show standard error of the mean. b) Weighting attributed to variables accounting for the top half of the variance from canonical analysis, see Table 6.5. Closed symbols indicate PLFA variables, open symbols indicate physical variables.

Table 6.6: Proportion of variance attributed to each variable of each primary canonical root. Most significant variables listed before dashed line.

$CR1_{(Physical)}$	% variance attributed to variable on CR1	$CR1_{(PLFA)}$
Sand (%)	94	
N (%)	78	
Loss on ignition (%)	76	
	74	16:1w7 c
TOC (%)	70	
TC (%)	62	
	58	17:0 isomer
CEC	50	
	50	19:2
	46	19:0 c
	46	18:1w9 t
	42	16:1w7 t
	34	18:1w9 c
	33	19:0
	33	17:1 isomer
C : N ratio	30	
	28	17:0
	26	ai15:0
	26	i16:0
	26	16:0
	25	20:0
	25	15:0
	20	18:2w6 c
	19	ai17:0
	18	i17:0
	13	14:1 isomer a
	13	i15:0
	11	15:1
Clay (%)	8.6	
	8.1	Me17:0 isomer
	7.6	18:0
	3.5	14:1 isomer b
pH	3.2	
	2.8	18:1w7 t
	2.6	18:0 isomer
	1.3	18:1 isomer
	1.2	17:0 c
	0.1	16:1 isomer

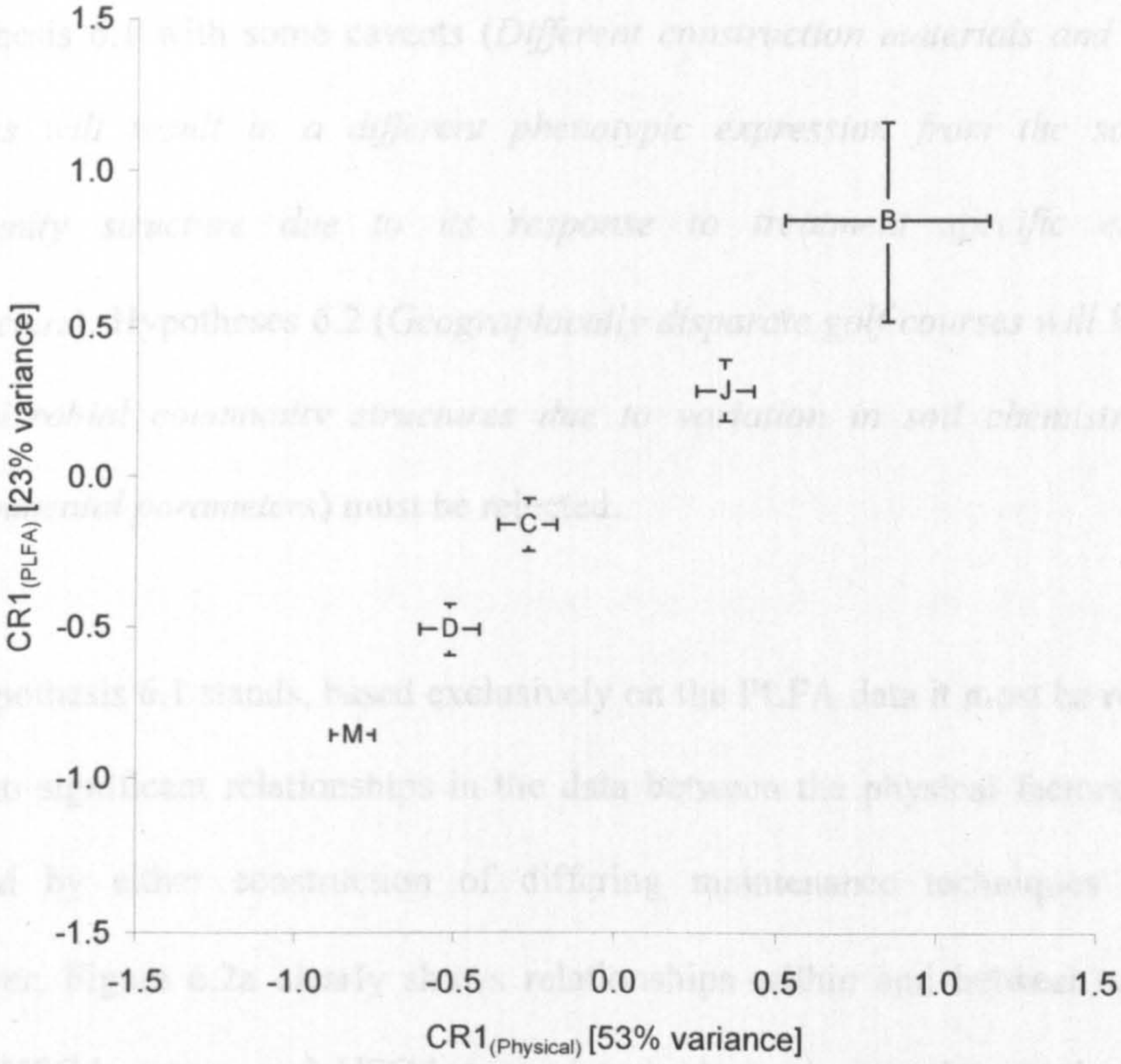


Figure 6.5: Canonical analysis of PLFA data and physical soil characteristics derived from five golf courses. a) Projection of mean co-ordinates of each canonical root (CR) with respect to Marquess (M); Dukes (D); John O'Gaunt (J); Carthagena (C); Buckingham (B) golf courses. Whiskers show standard error of the mean, n=15.

6. 4. Discussion

Analysis of the same data with different statistical methods leads to distinctly different conclusions. When the data are analysed by PCA significant treatment differences with regards to community structure are masked as the community does not interact with single physicochemical variables at a time, rather the interaction within and between these variables. When analysis using CCA is carried out, which allows for the analysis of both the physicochemical parameters alongside the microbial community structure distinct treatment differences become apparent, and, as such, allow justification for the acceptance of the hypothesis that might otherwise be rejected.

Analysis of PLFA biomarkers in these experiments using PCA alone leads us to accept Hypothesis 6.1 with some caveats (*Different construction materials and maintenance regimes will result in a different phenotypic expression from the soil microbial community structure due to its response to treatment specific environmental parameters*). Hypotheses 6.2 (*Geographically disparate golf courses will have different soil microbial community structures due to variation in soil chemistry and other environmental parameters*) must be rejected.

As Hypothesis 6.1 stands, based exclusively on the PLFA data it must be rejected, there were no significant relationships in the data between the physical factors which were affected by either construction of differing maintenance techniques (Table 6.5). However, Figure 6.2a clearly shows relationships within and between surfaces ($p < 0.01$). USGA greens and USGA topped tees show similar phenotypic communities irrespective of their geographical location thus suggesting that there was a distinct and coherent community structure that was always associated with USGA rootzone. Work carried out by the United States Golf Association also implies this relationship. A study using agar enriched colony counting in combination with direct soil DNA extraction showed that soils collected from golf courses in the United States of America were dominated by Gram positive bacteria. The USGA rootzone they tested contained principally Gram negative bacteria (Karp and Nelson 2004). These findings are reflected in results for these UK soils, as shown in Figure 6.2b. The PLFA's that were negatively loaded, causing the discrimination of USGA greens and USGA topped tees in Figure 6.2a were Gram negative community biomarkers. Community biomarkers

acting in the opposite direction, representing the golf course soils were indicative of Gram positive biotic associations.

Figure 6.2a also shows other relationships between soil microbial community structure and surface construction. The temporary greens were significantly different from all other surfaces ($p < 0.01$). This can be attributed to the way in which this green was both constructed and maintained. These greens were made by cutting areas of the fairway very closely (circa 5 mm), over-seeding with fine leaved grasses and considerable chemical intervention both to promote growth and prevent grass diseases, using fertilisers and fungicides. Once suitable for play, these greens were then maintained in this way for 12 months. Changes in vegetation management in agricultural systems, such as radically altering cultivation practices, have been shown to have significant impacts on their associated microbial community (Hedlund 2002; Clegg 2006). These workers' findings make associations between fairways, standard tees and standard greens challenging to explain. Separate groups would be expected in (at least) PC1 as the fertiliser, chemical and maintenance inputs were considerably different with greens receiving the most attention and fairways the least (see Figure 6.2a). These similarities might be explained as follows:

1. Any significant differences in phenotypic expression of microbial community may be predominantly associated with the roots and thatch present both in and on the soil. This portion of the soil was discarded to prevent potential contamination with plant roots (Section 6.2.1).
2. While the phenotypic structure of the community associated with each fairway, standard tee and standard green were not significantly different, the management

differences could potentially manifest themselves with respect to the community size (a parameter which was not be measured using this method of PLFA analysis). Differences in size and activity were shown by Karp & Nelson (2004) between USGA rootzones and golf course soils.

3. Interactions between the microbial community and the soil in which they live was governed by more factors than can be described using PCA followed by multiple linear regressions using general linear models. For this reason data was re-analysed using CCA and thus including physicochemical variables in the analysis rather than using them as part of a *post-hoc* test (see Section 6.2.2).

Hypothesis 6.2 was rejected because whilst there were statistically significant clusters of data associated with PC1 in Figure 6.3a there were no causal relationships with geographical location. This would imply that there was no relationship between the soil microbial community and physicochemical differences between surfaces. Superficially, the significantly different clusters of data points ($p < 0.01$) were arranged by age of golf course, with the newest courses being negatively weighted and the oldest being positively weighted. There was however no conclusive way of testing this relationship and no causal mechanism was evident from the projection of loading values (Figure 6.3b).

Further interpretations of the relationships between phenotypic microbial community structure and physicochemical soil parameters was resolved using CCA. Based on the analysis of this data using CCA, Hypothesis 6.1 (*Different construction materials and maintenance regimes will result in a different phenotypic expression from the soil*

microbial community structure due to its response to treatment specific environmental parameters) is accepted. All of the surfaces containing USGA rootzone group at the negative extreme of this analysis, with sand (%) and 16:1ω7 (an indicator of communities dominated by Gram-negative bacteria) being responsible for these trends. These findings are broadly in line with those of Karp and Nelson (2004). Steenweth *et al.* (2002) have also investigated physicobiological interactions of microbes in pasture and agricultural soils using CCA. They conclude that non-native grass lands were associated with unique microbial communities. This effect was demonstrated here with USGA greens and USGA topped tees being manipulated to maintain a dominant grass community of *Festuca* spp and *Agrostis* spp grasses rather than the ecologically successive grass (*Poa annua*).

In this analysis, the temporary greens were at the positively loaded extreme: the interpretation here was as with the PLFA data alone. The construction and maintenance of these surfaces significantly affects the soil microbial community. The loadings seen in Figure 6.4b confirm the suggested mechanism for these differences, with the weightings being positively drawn by % N and % TOC. An imbalance in these two variables has been shown to lead to the development of thatch, measured as percentage weight loss on ignition (Randell *et al.* 1972; Hope 1990; Perris 1996).

The inclusion of these physicochemical factors also resolved differences between fairways, standard tees and standard greens. This analysis indicates that standard greens were distinct from standard tees and fairways ($p < 0.05$). This separation reflects the differences in both chemical and management inputs to these surfaces as has been

shown in other grasslands (Hedlund 2002; Clegg 2006). Management practices such as top dressing with sandy materials in order to improve drainage and soil aeration (Hope 1990; Perris 1996a) had a clear influence on the phenotypic expression of the soil microbial community. The standard tees and fairways were not significantly different ($p > 0.05$) therefore any differences in nutrient input, through the increased frequency of mowing and any other management techniques, did not affect the phenotypic structure of the microbial community within the soil. The physical construction of the soil profiles of the fairway and the tee were very similar. The tee is essentially an area of the fairway that has been consolidated to increase bulk density and the turf improved by close management of the grass species present. The tee also receives a higher proportion of player traffic per unit area than the fairway (Perris 1996b). Despite these differences the composition of the nutrient inputs and the physical stresses are roughly equivalent on these two surfaces, which may account for the lack of significant difference in the phenotypic microbial community structure. These findings were consistent with other findings of workers where PLFA analysis failed to detect any differences in community structure in pasture lands at different geographical locations and where the time since last tillage operation varies (Steenwerth *et al.* 2002). The suggested mechanism for this was that the effect that grass growth has on the soil microbial community is rapid, consistent and long-lasting.

The mean variance associated with the physical variables was significantly greater than that with the PLFA variables ($p = 0.03$), this result reflects the composition of the nine individual variables accounting for more than 50% variance on CR1 (see Table 6.5). Two-thirds of these variables were physicochemical measurements with sand (%)

dominating the variance within the experiment. This shows that physicochemical factors were the most important in affecting the phenotypic expression of the microbial community within this sand dominated soil. Goodfriend (1998) studied sand dune and spatially related agricultural soils in both Mexico and the USA using the Biolog™ technique to characterise microbial communities. She concluded that the relationships between the microbial communities from eight different soils reflect similarities in habitat type more closely than geographical location, which is associated with the phenotypic expression. The physical environment associated with the construction and management of different surfaces on geographically separated golf courses were more important in dictating the microbial community structure than the geographical location alone as each surface associated community identified in Figure 6.4a was found at one or more golf courses studied in this experiment.

A wide body of evidence indicates that environmental pH has a significant impact on both species community structure of both micro and macro organisms found within the soil (Edwards and Lofty 1977; Hope 1990; Edwards 1996; Perris 1996b; Doube and Brown 1998; Kretzschmar 1998; Lavelle 2001; Baker and Whitby 2003). Saleh-Lakha *et al.* (2005) highlight that it is variation in soil pH and other physicochemical factors which affects gene expression from bacteria thus directly affecting the community structure as measured by PLFA. The composition of the dominant soil microbes within the system are strongly influenced by the environment in which they reside. Further to this recent research has shown that species richness of soil microbial communities are intimately related with soil pH, independent of geographical location at a continental scale (Fierer and Jackson 2006). The data in Table 6.5 would initially appear to

contradict the findings of Fierer & Jackson (2006), the proportion of variance attributed to pH in this analysis was very small, 3.2%, meaning it was an insignificant variable in this analysis. A close inspection of both sets of results shows that the pH of the soils in the golf course system fall into a very narrow range (mean pH = 6.3, ± 0.15 at 95% confidence) where as the survey conducted by Fierer & Jackson (2006) have a pH range from 3.5 to 9. Any relationship between golf course soils microbial communities and pH from this study was therefore likely to be obscured by the narrow soil pH range represented.

When the physicochemical factors were considered the microbial community associated with each golf course were significantly different ($p < 0.01$). Golf courses that were geographically close to each other were also proximal in the CCA (Figure 6.5). The courses at Woburn Golf and Country Club were next to each other, followed by the courses at John O'Gaunt Golf Club followed by the single course at Buckingham. The increased error associated with the samples from Buckingham was attributed to the inclusion of the heavily negatively weighted USGA green samples within this treatment. This indicated, based on the evidence derived from CCA Hypothesis 6.2 must be accepted (*Geographically disparate golf courses will have different soil microbial community structures due to variation in soil chemistry and other environmental parameters*). Each grouping of golf course data points in Figure 6.5 contains a combination of at least three surfaces, therefore it must be differences in the local environment that caused this effect. Management by different individuals can be ruled out as an unaccounted for variable as these courses (both courses at John O'Gaunt Golf Club) did not form associations in the CCA (Figure 6.5).

Based on these findings it is possible to draw wider conclusions than Goodfriend (1998); irrespective of variation in geography and individual management strategies for different turf surfaces on a golf course, the microbial community structure appears to reflect similarities in the physicochemical component of the habitat type. The microbial community associated with two golf greens (or any other golf course surface studied here) that have been constructed and managed as per industry standards (Hope 1990; Perris 1996b) will be similar. The data also highlights the fact that care must be taken when using the PLFA technique for inter-site analysis. By design the technique is a phenotypic measurement of the soil microbial community.

Chapter 7: Intra-course microbial analysis: A comparison of microbial community size and structure on tees, fairways and greens

7. 1. Introduction

Earthworm ecology is intrinsically linked to soil microbial ecology. The soil microbial community represents the available food source to earthworms through both direct ingestion of microbes and through gut absorption of exudates that the soil microbes produce. Understanding the differences in microbial size and community structure on different areas of golf courses can be used to assist in the interpretation of earthworm activity in these areas. Only a limited amount of soil microbial ecology has been carried out to investigate inter-course differences in the size and structure of microbial communities at different golf courses (Chapter 6; Hagley 2002; Karp and Nelson 2004). Even less work focussing on intra-course differences has been published. Intra-course differences can be divided into two groups; differences in microbial community in relation to different surfaces that are maintained as parts of golf courses and differences in microbial community size and structure with depth through the soil profile. Some work has been carried out pertaining to the effects of depth in the soil profile on microbial community (*personal communications; S. Jeffery* and Zvyagintsev 1994; Fritze *et al.* 2000; Petsch *et al.* 2003; Fierer *et al.* 2003). However, the majority of research focuses on the top 250 mm of the soil profile as a bulked sample. On golf courses where nitrogen fertilisers are intensively used and mowing heights are low, a large amount of root and crown material develops to form thatch. This material is rich in microbial biomass and so provides an ideal food source for the earthworm community. Research has shown that earthworms are intimately involved in the redistribution and breakdown of thatch while feeding through this horizon of the soil (Randell *et al.* 1972; Potter *et al.* 1990).

Data presented in Chapter 6 using canonical correlation analysis indicates that microbial physicobiological interactions in golf courses are dominated by the physical factors in the environment. The physicochemical parameters of the soil are different in each horizon of the soil profile. In pedological terms, the thatch layer found on golf courses is described as an Ah horizon, a feature that is more frequently associated with woodland and uncultivated soils (Hodgson 1997). On a golf course this thatch layer can extend to a considerable depth (up to 40 mm on surveyed golf courses, personal observations) and thus presents an environment which is not usually associated with grassland pedology (or ecology). Hence golf course soils have a plentiful supply of food in the soil matrix from which earthworms can feed. Analysis carried out in Chapter 6 using PCA also showed that there were no significant differences in microbial community structure between tees, fairways and greens based on a bulked soil core to a depth of 225 mm. This suggests that if treatment differences do exist then they may be confined to a relatively shallow depth from the surface where the principal effects of any management strategies are realised.

Investigations into the relationships between depth and microbial community structure on each playing surface of a golf course are therefore pertinent in characterising the environments in which earthworms live and thrive, determining if treatment differences are localised in the top of the profile of each surface.

The following hypotheses will be tested:

Hypothesis 7.1. There is a significantly different microbial biomass carbon and phenotypic microbial community structure in the 0 - 75, 76-150 and 151-225 mm

depth bands of the soil profile on all managed areas of a golf course, as a consequence of the different management strategies applied to these surfaces.

Hypothesis 7.2. There is a significantly different phenotypic microbial community structure in the 0 – 75 mm layer of the soil profile between areas of a golf course as a result of management in different ways for use as tees, fairways and greens when compared to the rough.

7. 2. Materials and methods

7. 2. 1. Experimental design

For this study, only the John O'Gaunt golf course at John O'Gaunt golf club was used due to practical restrictions on the number of samples that could be analysed. This course was selected as it is the oldest complete course of the five studied throughout this body of work. Differences between the surfaces of the course would be expected to be best developed here.

Four surface types (in statistical terms, treatments) were identified and were given the following designations:

- Tee (designated T)
- Fairway (designated F)
- Green (designated G)
- Rough (designated R)

The rough was included in this investigation because it was the closest representation of natural grassland for the region of the course.

Five randomly selected holes were used as replicates per surface (Figure 7.1). At each sample site five soil cores (10 mm diameter, 225 mm depth) were taken within a 1 m^2 quadrat randomly placed on the surface. Each core was divided into 0-75 mm, 76-150 mm and 151-225 mm layers from the grass surface and sealed in a separate bag (n = 60). No plant matter was removed from the samples in the field.

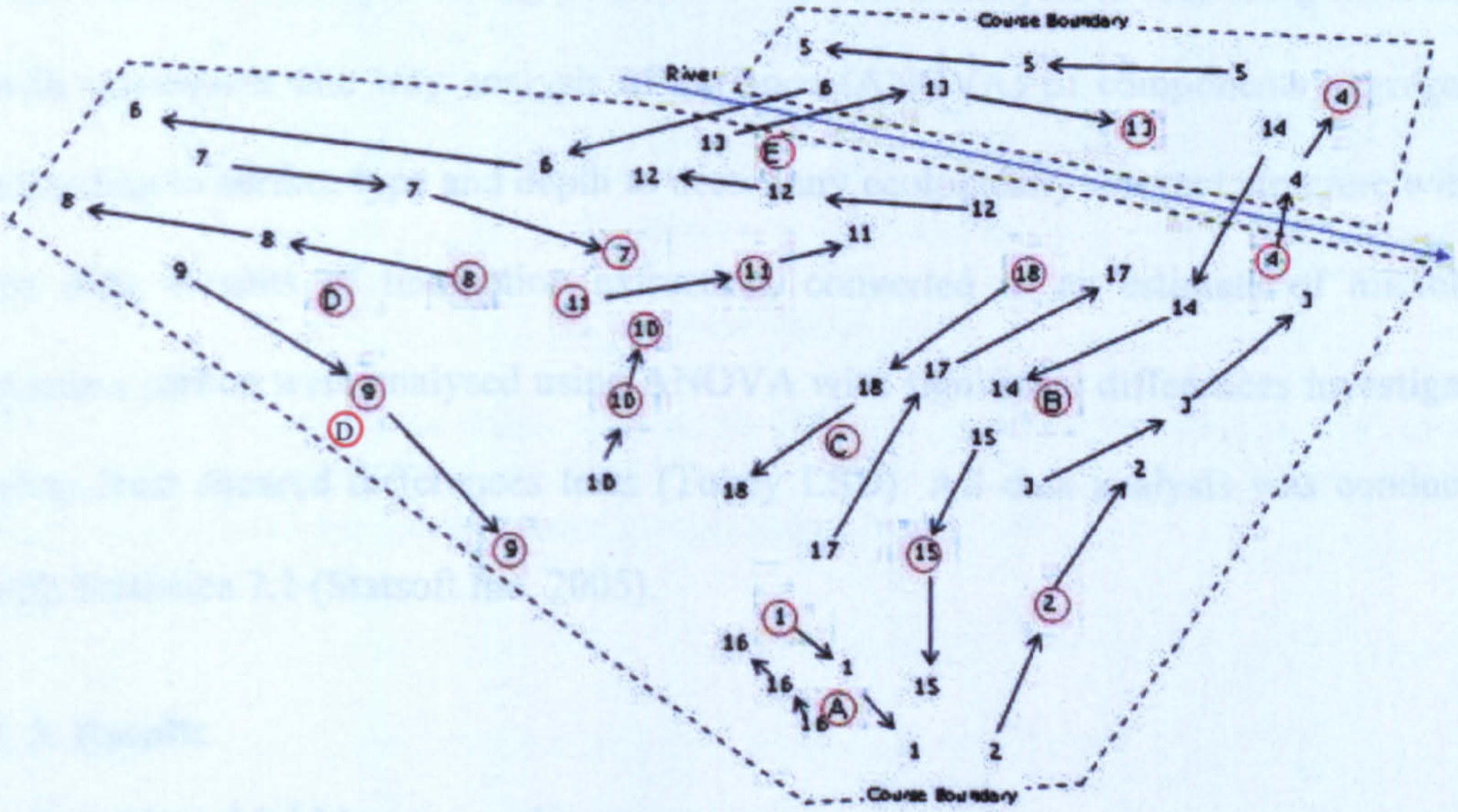


Figure 7.1: Location of sampling points at John O'Gaunt golf club, numbers denote holes. Circled numbers denote surfaces sampled. Letters indicate areas of rough sampled. Arrows show direction of play from, tee to centre of fairway to green. Not to scale.

7. 2. 2. Laboratory analyses

Samples were stored for 7 days at 4°C prior to homogenisation at field moisture content to pass through a 2 mm sieve. This sieve size was selected to exclude the maximum amount of plant matter. From each depth layer sample, one third (by weight) of the sample was pooled and homogenised to provide a repeat of the analysis reported in Chapter 6 for this golf course. This resulted in four different layers of the soil profile for each treatment.

Each of these samples was divided into two portions and half was snap-frozen at

-86°C and then freeze dried using an Alpha 1-2 LD freeze dryer (Christ Freeze Driers, Osterode-an-Harz, Germany) after which phenotypic microbial community structure was analysed by PLFA analysis, as described in Chapter 2.7. The remaining soil was used to calculate microbial biomass carbon as described in Chapter 2.6.

7. 2. 3. Data analysis

PLFA data were analysed using principal components analysis (PCA) using covariance with subsequent one way analysis of variance (ANOVA) of components aggregated according to surface type and depth to detect any ecologically relevant structure within the data. Results of fumigation extraction, converted to an estimate of microbial biomass carbon were analysed using ANOVA with significant differences investigated using least squared differences tests (Tukey LSD). All data analysis was conducted with Statistica 7.1 (Statsoft Inc. 2005).

7. 3. Results

7. 3. 1. Microbial biomass carbon

Microbial biomass carbon concentrations were significantly greater in the 0-75 mm layer than those below this depth ($p < 0.01$; Figure 7.2). The microbial biomass associated with the laboratory pooled sample confirms the fidelity of the assay (Figure 7.2).

Significant differences were also evident between surface types ($p < 0.01$). Two distinct groups are identified: fairway and rough, and tee and green. Microbial biomass carbon was almost two-fold greater in the fairway and rough than that found in the tee and green (Figure 7.3).

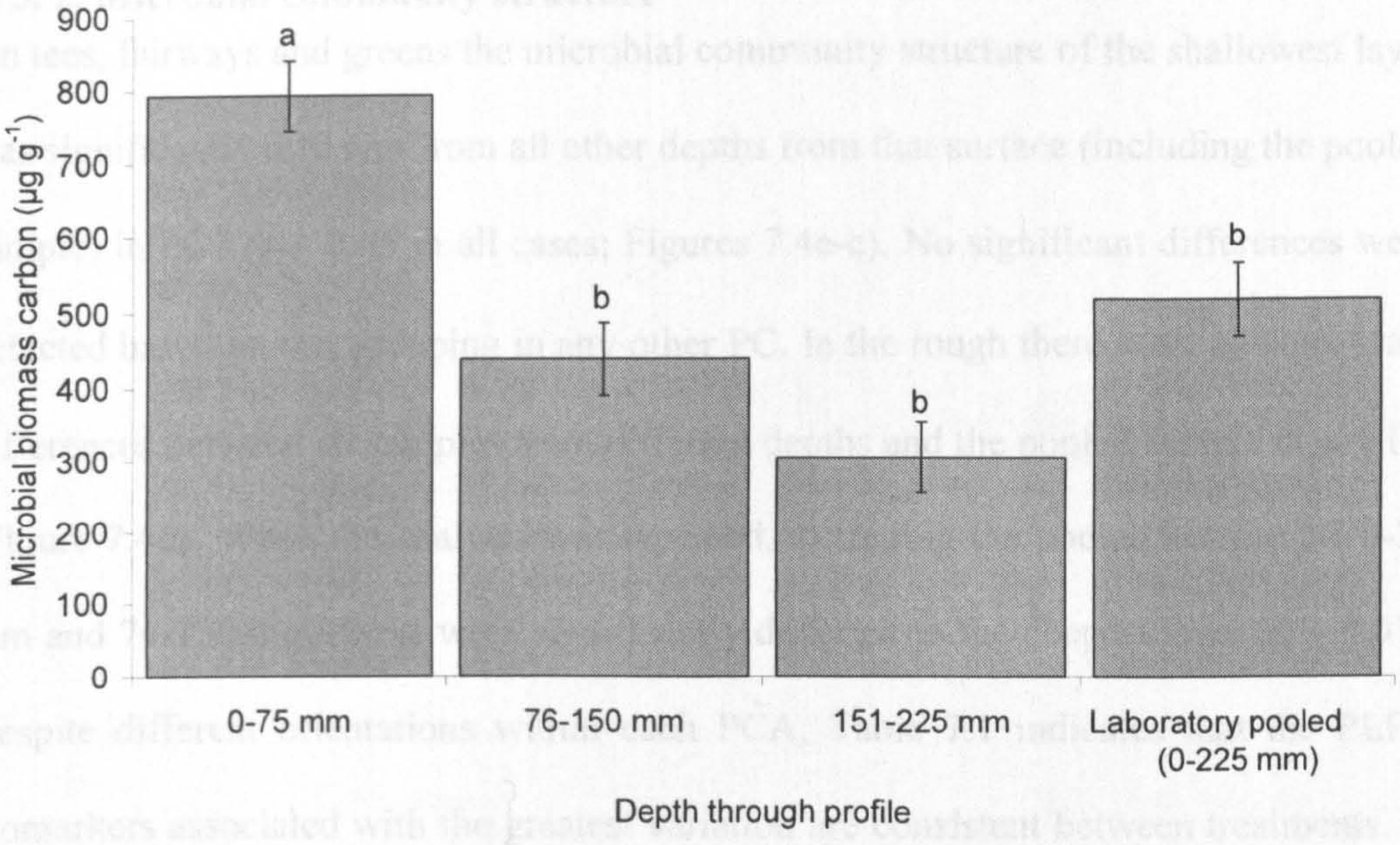


Figure 7.2: Microbial biomass carbon at different depths through the soil profile averaged across all surfaces at John O'Gaunt golf club. Whiskers show pooled standard error. Letters indicate heterogenous groups using Tukey LSD.

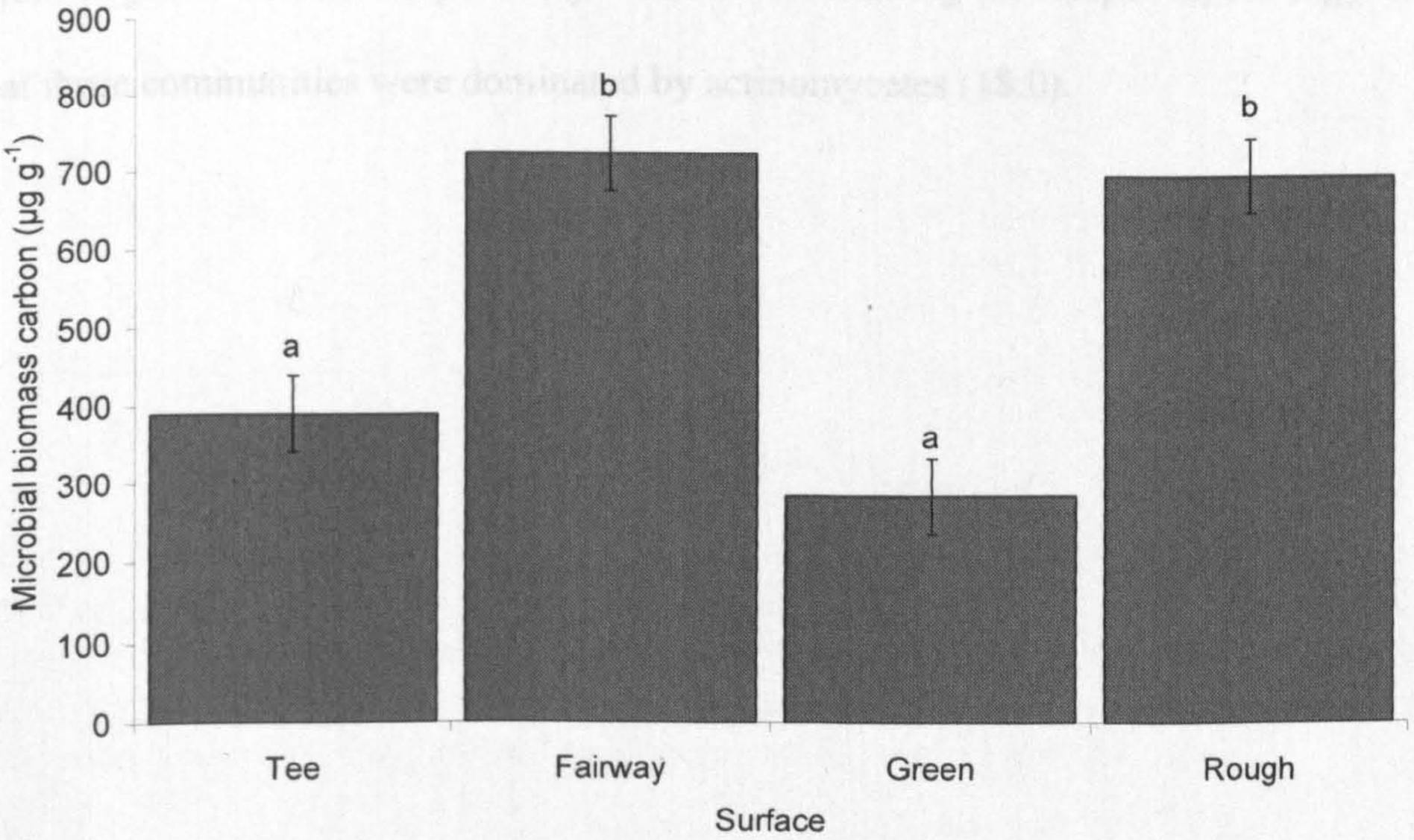


Figure 7.3: Microbial biomass carbon from pooled samples taken from all surfaces at John O'Gaunt golf club. Whiskers show pooled standard error. Letters indicate heterogeneous groups using Tukey LSD.

7. 3. 2. Microbial community structure

On tees, fairways and greens the microbial community structure of the shallowest layer was significantly different from all other depths from that surface (including the pooled sample) in PC1 ($p < 0.05$ in all cases; Figures 7.4a-c). No significant differences were detected based on this grouping in any other PC. In the rough there were no significant differences between all samples from different depths and the pooled sample in any PC (Figure 7.4d). When the analysis was repeated, excluding the pooled sample the 0-75 mm and 76-150 mm layers were significantly different to the deepest layer ($p < 0.01$). Despite different orientations within each PCA, Table 7.1 indicates that the PLFA biomarkers associated with the greatest variation are consistent between treatments. In the shallowest layer the PLFAs 16:0 and 18:1ω9 c are predominantly recorded (Figure 7.5). Individually they are acknowledged to be indicative of Type I methanotrophs and Gram negative bacteria respectively. PLFAs representing the deeper layers suggested that these communities were dominated by actinomycetes (18:0).

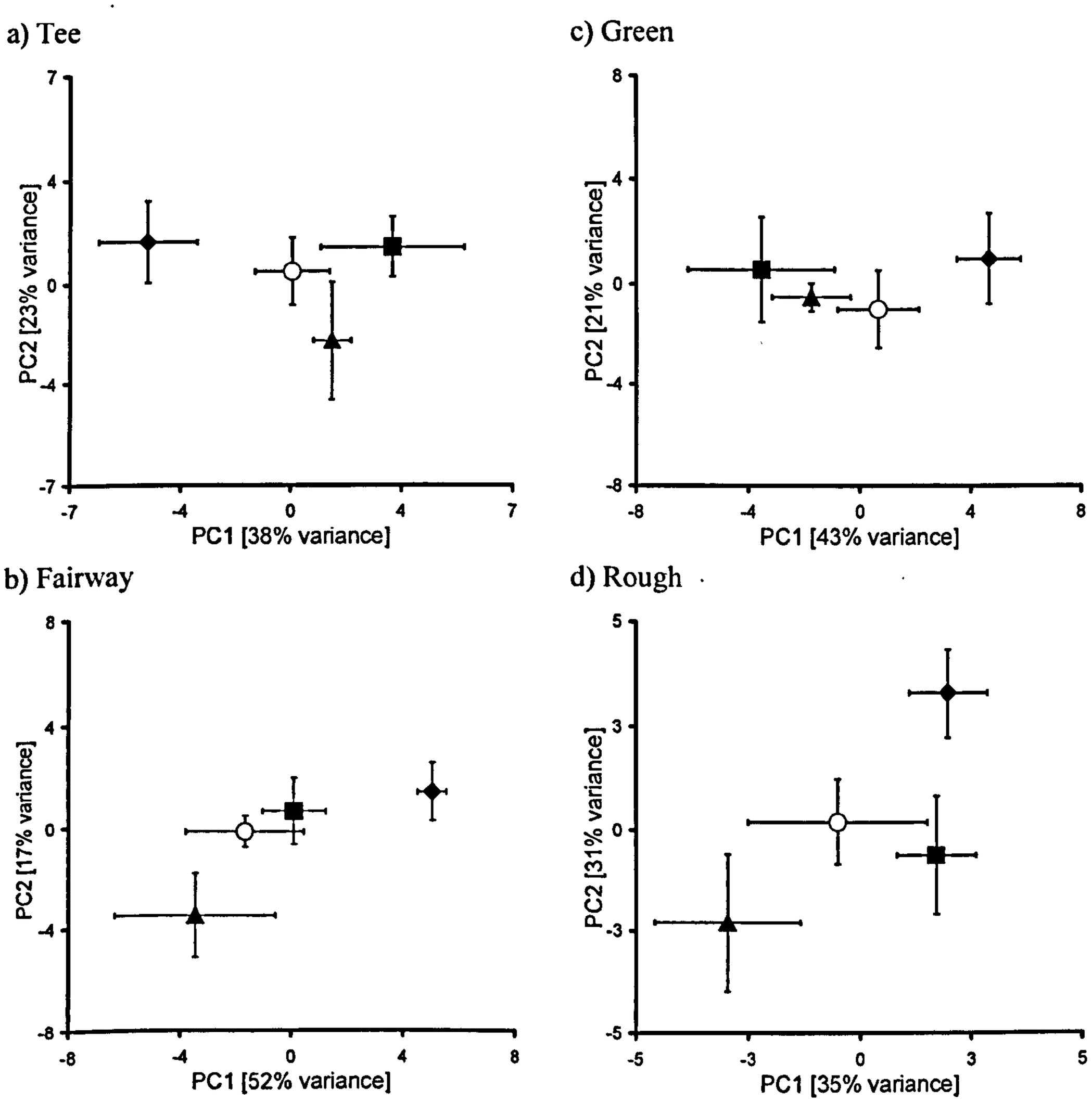


Figure 7.4: Principal component (PC) analysis of PLFA data derived from John O'Gaunt golf course. Projections show mean co-ordinates of PC1 and PC2 with respect to different layers through profile. 0-75 mm (♦); 76-150 mm (■); 151-225 mm (▲); Pooled 0-225 mm (○) for a) Tees; b) Fairways; c) Greens; d) Rough. Whiskers show standard error of the mean. Loading values shown in Table 7.1.

Table 7.1: PLFA biomarkers ranked by loading attributed to PC 1 in separate PCA, by surface. Frequently heavily weighted biomarkers with relation to differences between layers are highlighted in bold.

T		F		G		R	
PLFA	Loading on PC 1	PLFA	Loading on PC 1	PLFA	Loading on PC 1	PLFA	Loading on PC 1
18:0	**1.13**	**16:0**	**2.90**	**16:0**	**2.97**	19:0 c	2.56
20:0	1.04	**18:1w9 c**	**1.91**	**18:1w9 c**	**2.43**	**18:1w9 c**	**1.44**
18:1 *i*	1.04	18:1w9 t	**0.98**	18:1w9 t	1.51	18:1w9 t	**1.19**
18:1w7 t	0.83	i15:0	0.68	19:0 c	0.98	19:2	0.22
19:0	0.70	18:2w6 c	0.51	18:2w6 c	0.42	18:1 iso	0.14
17:1 iso	0.51	i16:0	0.39	16:1w7 c	0.41	18:0 iso	0.13
19:2	0.47	ai15:0	0.34	i15:0	0.25	18:2w6 c	0.13
18:0 iso	0.33	16:1w7 c	0.32	17:0 c	0.19	Me17:0 iso	0.11
19:0 c	0.30	17:0 iso	0.16	15:0	0.04	18:1w7 t	0.03
15:1	0.26	17:0 c	0.12	i17:0	-0.08	i17:0	-0.09
16:1w7 t	0.26	15:0	-0.01	ai15:0	-0.14	15:0	-0.12
17:0 iso	0.23	i17:0	-0.05	ai17:0	-0.16	20:0	-0.14
17:0	0.23	ai17:0	-0.05	Me17:0 iso	-0.18	17:1 iso	-0.14
16:1 iso	0.18	14:0	-0.11	19:2	-0.18	17:0 iso	-0.15
i17:0	0.12	19:2	-0.17	17:0	-0.19	17:0	-0.16
ai17:0	0.11	17:0	-0.18	i16:0	-0.20	16:1 iso	-0.19
Me17:0 iso	0.11	18:0 iso	-0.24	18:0 iso	-0.24	17:0 c	-0.19
14:0	0.05	18:1 iso	-0.26	14:0	-0.25	14:0	-0.19
14:1 iso(a)	0.04	14:1 iso(b)	-0.26	17:0 iso	-0.25	14:1 iso(b)	-0.19
i16:0	0.03	14:1 iso(a)	-0.27	14:1 iso(b)	-0.32	16:1w7 c	-0.21
14:1 iso(b)	0.01	16:1 iso	-0.27	16:1 iso	-0.33	15:1	-0.23
15:0	0.00	17:1 iso	-0.31	18:1w7 t	-0.34	ai17:0	-0.24
17:0 c	-0.09	16:1w7 t	-0.37	14:1 iso(a)	-0.41	14:1 iso(a)	-0.30
18:2w6 c	-0.30	Me17:0 iso	-0.40	15:1	-0.43	i15:0	-0.32
ai15:0	-0.37	18:1w7 t	-0.40	17:1 iso	-0.48	i16:0	-0.34
i15:0	-0.72	15:1	-0.43	16:1w7 t	-0.51	ai15:0	-0.40
16:1w7 c	-0.94	19:0	-0.62	18:1 iso	-0.78	19:0	-0.43
18:1w9 c	**-1.33**	20:0	-0.70	19:0	-0.93	16:1w7 t	-0.48
18:1w9 t	-1.67	19:0 c	-0.72	20:0	-0.98	**16:0**	**-0.65**
16:0	**-2.56**	**18:0**	**-2.49**	**18:0**	**-1.83**	**18:0**	**-0.77**

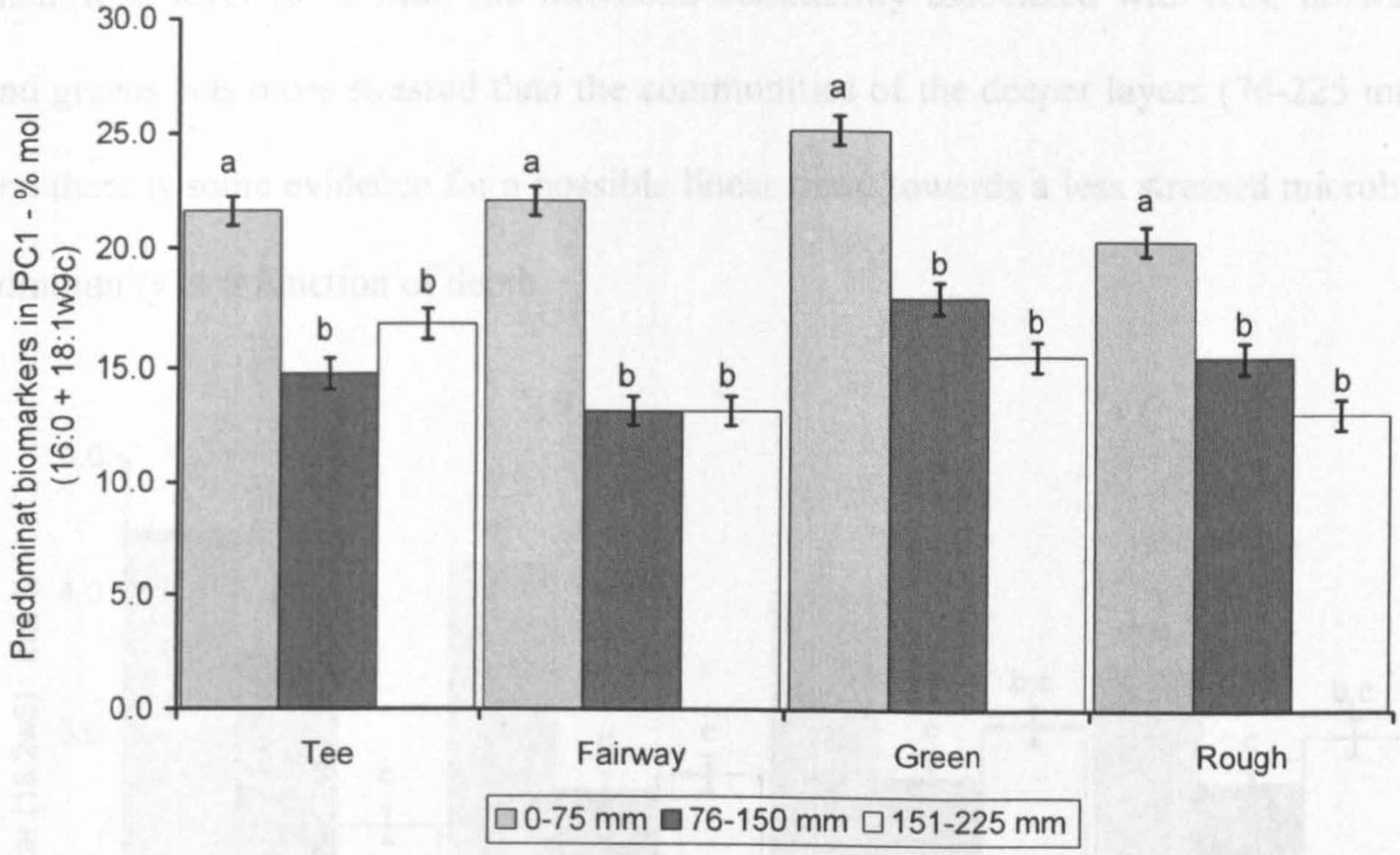


Figure 7.5: Variation in predominant biomarkers from PC1 ($\Sigma_{16:0+18:1\omega9\ c}$) in different depth layers. Whiskers show pooled standard error. Letters indicate heterogeneous groups identified using LSD in different depth layers.

One way analysis of individual biomarkers can help determine interactions within the microbial community. The biomarker 18:2ω6 is typically an indicator of eukaryotes, particularly fungi. Decreasing relationships with the proportion of this biomarker and depth through the profile in the tee, fairway and green were evident ($p < 0.05$; Figure 7.6). There was a greater abundance of 18:2ω6 in the shallowest layer (0-75mm) in these treatments than in both layers below it (76-225 mm). This relationship was not evident in roughs, where there was no significant difference between different concentration of 18:2ω6 (% mol) in each layer ($p > 0.05$).

An indication of the environmental (temperature and water) stress can be derived from the ratio of trans 16:1ω7 to cis 16:1ω7 (Kieft *et al.* 1994). Figure 7.7 shows clear trends in this stress response with depth through the soil profile ($p < 0.05$). In the

shallowest layer (0-75 mm) the microbial community associated with tees, fairways and greens was more stressed than the communities of the deeper layers (76-225 mm) and there is some evidence for a possible linear trend towards a less stressed microbial community as a function of depth.

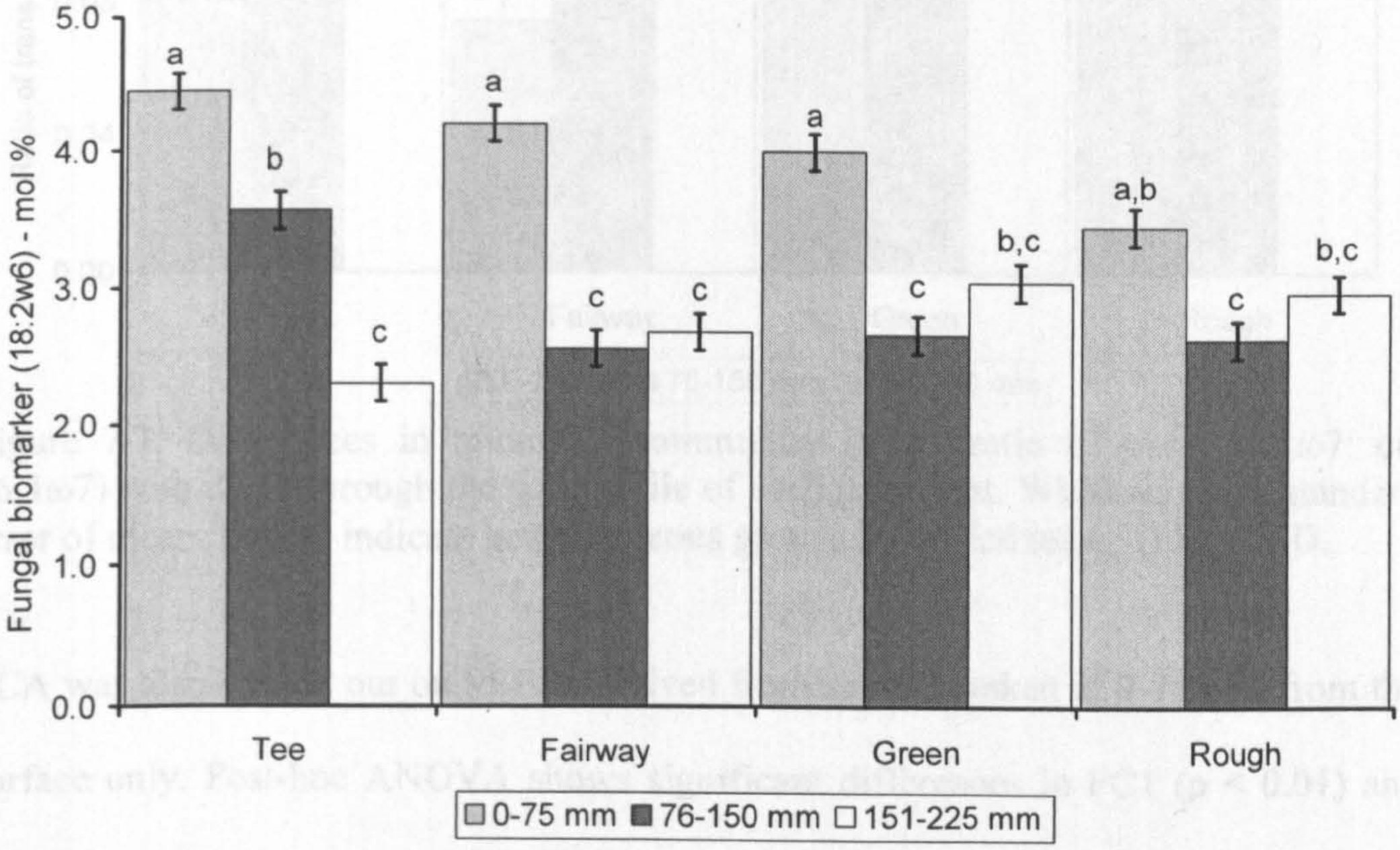


Figure 7.6: Variation in proportion (mol %) of fungal biomarker (18:1ω6) at different depths, and on different surfaces as a single variable used in the PCA of PLFA data. Whiskers show pooled standard error. Letters indicate heterogeneous groups identified using Tukey LSD.

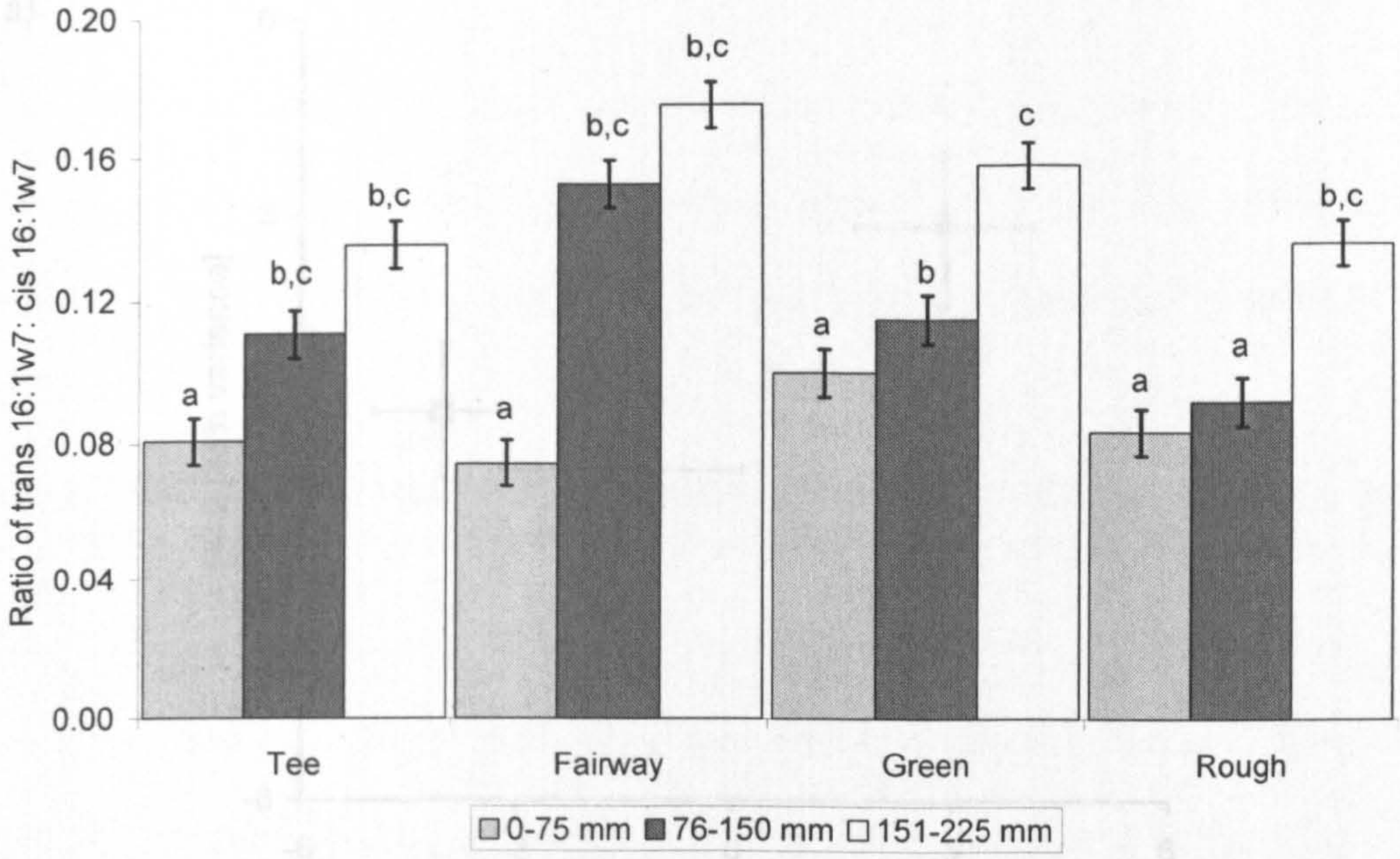
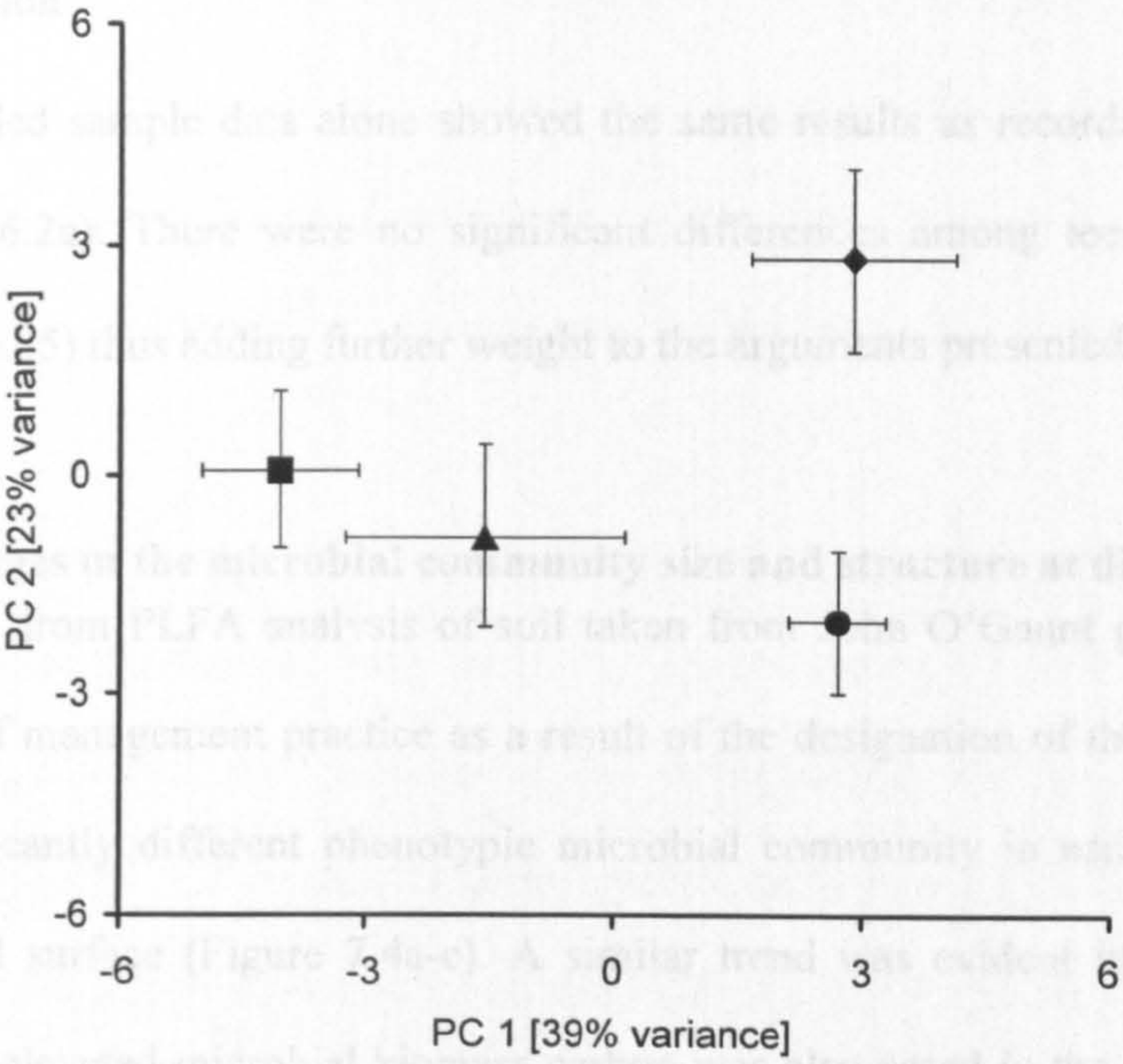


Figure 7.7: Differences in microbial community stress (ratio of trans 16:1ω7: cis 16:1ω7) with depth through the soil profile of each treatment. Whiskers show standard error of mean. Letters indicate heterogeneous groups identified using Tukey LSD.

PCA was also carried out on PLFAs derived from samples taken at 0-75 mm from the surface only. Post-hoc ANOVA shows significant differences in PC1 ($p < 0.01$) and PC2 ($p < 0.05$). This analysis indicates that tees and greens were distinct from each other and from fairways or roughs in this layers community structure (Figure 7.8a). The biomarkers which were pulled in three directions are indicative of communities from each surface. Microbial communities dominated by Type I methanotrophs (16:0), connected with greens; Gram negative bacterially dominated communities (18:1ω9 t and 18:1ω9 c), related with tees; and communities rich in anaerobic Gram positive bacteria (*cyc*19:0) associated with fairways and roughs.

a)



b)

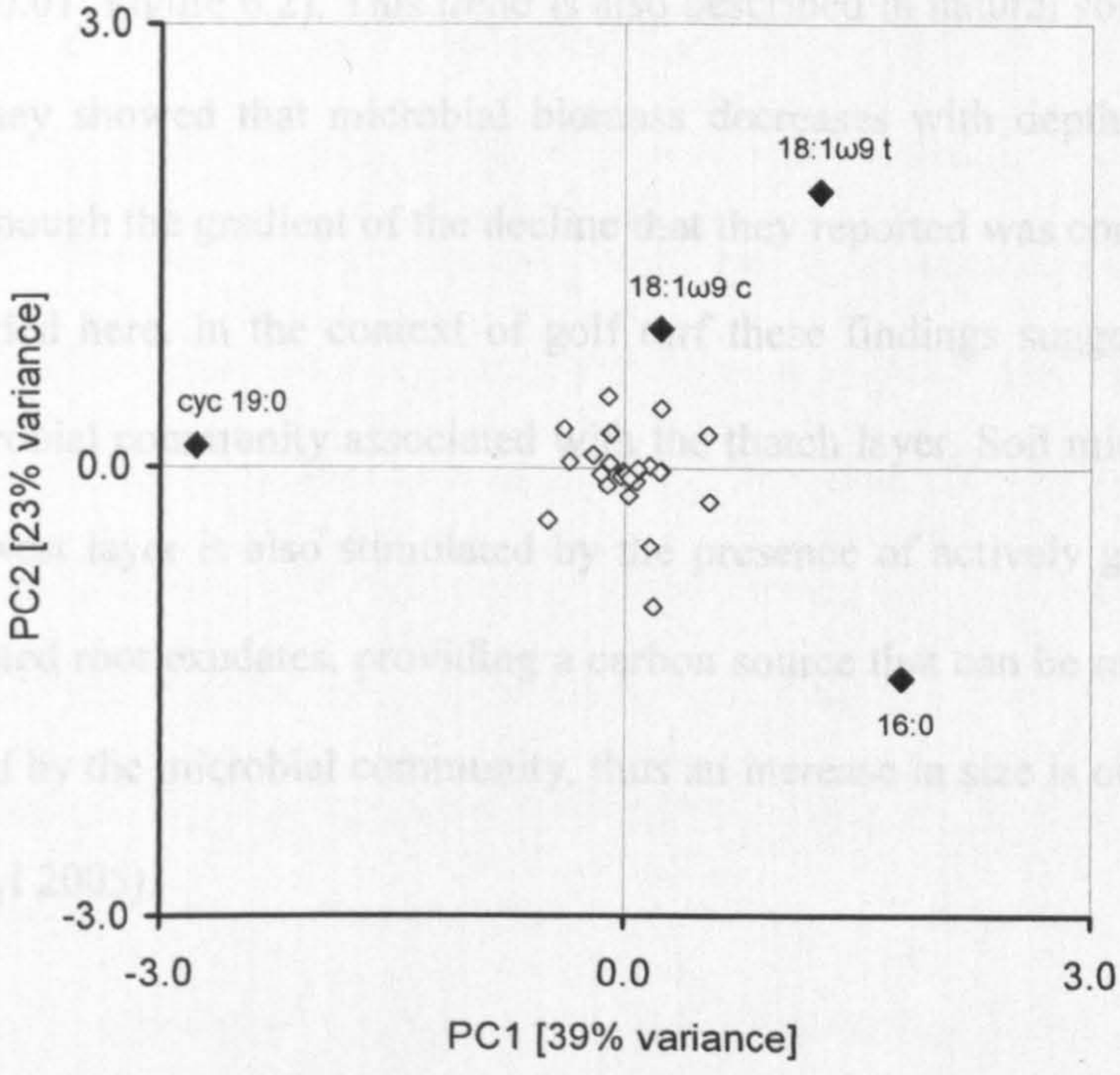


Figure 7.8: Principal component (PC) analysis of PLFA data derived 0-75 mm depth band on John O'Gaunt golf course. a) Projections shows mean co-ordinates of PC1 and PC2 with respect to greens (●), fairway (▲), tee (■) and rough (♦).Whiskers show standard error of the mean (n=5). b) Loading values associated with PC1 and PC 2. Selected loading values attributed to significant differences between surfaces projected in Figure 7.8a marked with a closed symbol.

7. 4. Discussion

PCA of pooled sample data alone showed the same results as recorded in Chapter 6 (see Figure 6.2a). There were no significant differences among tees, fairways and greens ($p > 0.05$) thus adding further weight to the arguments presented in Section 6.4.

7. 4. 1. Changes in the microbial community size and structure at different depths

Data derived from PLFA analysis of soil taken from John O'Gaunt golf club shows that each turf management practice as a result of the designation of the Rules of Golf had a significantly different phenotypic microbial community in each 75 mm layer from the soil surface (Figure 7.4a-c). A similar trend was evident in the rough. A significantly elevated microbial biomass carbon was also noted in the 0-75 mm depth band ($p < 0.01$; Figure 6.2). This trend is also described in natural soils by Fierer *et al.* (2003). They showed that microbial biomass decreases with depth through the soil profile, although the gradient of the decline that they reported was considerably steeper than recorded here. In the context of golf turf these findings suggest that there is a larger microbial community associated with the thatch layer. Soil microbial activity in the shallowest layer is also stimulated by the presence of actively growing roots and the associated root exudates, providing a carbon source that can be rapidly assimilated and utilised by the microbial community, thus an increase in size is observed (Agbenin and Adeniyi 2005).

It is possible for the fumigation-extraction procedure to over-estimate the microbial biomass carbon present due to contamination from other cellular sources (e.g. plants). Each sample was homogenised to pass through a 2 mm sieve and all visible plant root matter was removed. It is therefore unlikely that these results are an artefact within the

data. Hypothesis 7.1 is therefore accepted (*There is a significantly different microbial biomass carbon and phenotypic microbial community structure in the 0 - 75, 76-150 and 151-225 mm depth bands of the soil profile on all managed areas of a golf course, as a consequence of the different management strategies applied to these surfaces*).

The mechanisms which result in these differences in community can be interpreted through the phenotypic community structure. Discrimination was only evident in PC1 between tees, fairways and greens ($p < 0.05$ in all cases). PLFAs attributed to the greatest loadings of PC1 in each of these separate analysis shows marked similarities (Table 7.1). Biomarkers associated with the 0-75 mm layer 16:0 and 18:1ω9 c) have been associated with algal biofilms of waste waters (Keinanen *et al.* 2002). Personal observations during a range of sampling on golf courses throughout the year has revealed the formation of green or black mats both in the soil profile and occasionally on the soil surface. If these observed biofilms were algal assemblages then an explanation of the recorded community structures in Figure 7.4a-d can be derived: On tees, fairways and greens were both organic and inorganic nitrogen and organic carbon are available in excess, through regular grass cutting and fertiliser applications the nutrient requirements for the growth of the soil biota are ideal. This coupled with regular irrigation results in a reduced water stress on any soil microbes. However short mowing heights means that the soil conditions are ideal for photoautotrophs. Investigations carried out by Jahnke and Priefer (2002) show that the colonisation and demise of soil algal biofilms are rapid, and that they are always found at or near the soil surface where light levels are at a maximum. These conditions have been reported at other golf courses and are frequently associated with bad drainage and slippery

under foot, resulting in dangerous play conditions. (*personal communications; Golf Course Managers*) The same soil micro-environment does not exist in the rough, where nutrient supply, irrigation and light penetration to the soil are all considerably lower than on the other areas of the golf course. This suggests that for the shallowest layer of rough these relationships would not be as strong, or may not exist at all (Table 7.1). To determine if this is the mechanism by which the community is different between the treatments a further experiment would be required. An investigation of the chlorophyll a content of the soil in the 0-75 mm layer, in comparison to deeper in the soil profile on each of these treatments would indicate if the mechanism suggested above is correct.

Kieft *et al.* (1994) showed that the change in ratio of PLFA's such as 16:1ω7 cis: 16:1ω7 trans could be used as indicators of stress through either starvation or desiccation. In the microbial communities measured during these investigations it is most likely significant differences in community scale stress are as a result of desiccation through increased evaporation of soil water and the uptake of water by plants through evapotranspiration. The shallowest layer of these samples is also characterised by indicators of a significantly larger fungal community ($p < 0.01$; Figure 7.7). This can be attributed to the fact that the surface layer of each treatment was dominated by thatch, generated through management activities. Thatch typically contains a high proportion of roots and root exudates, these complex sugars represent ideal nutrients for fungal growth hence a larger proportion of the microbial community is fungal, although bacteria will also be present in significant concentrations.

Bacterially dominated communities (18:0) were implicated by the loading values for PC1 in the deeper sample layers in all treatments (Table 7.1). The trend towards bacterial domination is also noted by Fierer *et al.* (2003) who found Gram negative bacteria and actinomycetes in increasing proportion at greater depths. This community was present deeper in the soil profile as the depth offers protection from potentially prolonged periods of desiccation and a wide range of intensity of UV irradiation to which these micro-organisms are sensitive. This microbial community structure was also capable of surviving in lower nutrient status soils, which were found as depth increased, a factor that can be related to the size of the microbial community; layers below the shallowest (0-75 mm) sample had significantly less microbial biomass carbon.

7. 4. 2. Differences between the microbial community size and structure on surfaces types

Analysis of each treatment showed that the 0-75 mm layer supported a significantly different microbial community when compared to deeper in the soil. When PCA was carried out on data from only this layer significant differences were recorded between surface types (Figure 7.5a; $p < 0.05$) not evident at the deeper layers. The intensity of management also appeared to affect microbial biomass, with highly managed surfaces (tee and green) distinct from the less managed surfaces (rough and fairway); Figure 7.3. This data supports Hypothesis 7.2 (*There is a significantly different phenotypic microbial community structure in the 0 – 75 mm layer of the soil profile between areas of a golf course as a result of management in different ways for use as tees, fairways and greens when compared to the rough*).

The values of microbial biomass carbon recorded for the areas of the golf course that receive comparable inputs to agricultural pastureland (rough and fairway) are comparable in terms of microbial biomass carbon typically found in such systems (Lovell *et al.* 1995; Bardgett *et al.* 1999; Bardgett and McAlister 1999). Bardgett *et al.* (1997) have also demonstrated that microbial biomass carbon of soils in unfertilised meadows is greater than adjacent fertilised grasslands. This effect is seen here: the most managed areas of the golf course support the lowest concentrations of soil microbial biomass. Additional inputs of inorganic fertiliser are high on tees and greens in order to produce the desired sward. Nutrient inputs are also provided to the soil biota from the regular cutting of the grass in these areas, even when the grass clippings are removed, due to the stimulation to plant and microbial resulting from physical disturbance. Greenhouse lysimeter trials on grassland systems have also shown that in soils which are disturbed, more organic C is leach than undisturbed systems (Matlou and Haynes 2006). Many of the turf maintenance practices e.g. hollow tine cultivation and top dressing result in considerable disturbance (Perris 1996a), and as such may reduce microbial biomass because of this.

Interpretation of differences in community structures based on the biomarkers highlighted through PCA of the 0-75 mm layer is as follows:

Greens: The biomarker found associated with the greens was 16:0, there are several conflicting reports in literature as to the community structure that this biomarker is indicative of. It has been associated with a wide range of bacterial communities (Pelz *et al.* 2001; Jones *et al.* 2003; Feng *et al.* 2003; Keinanen *et al.* 2003; Knief *et al.*

2006). The environmental drivers on golf greens suggest that the most likely candidate in this situation is as an indicator of communities dominated by Type I methanotrophs (Zelles *et al.* 1992). In the surface layer of the green, which is frequently saturated through irrigation, the CH_4 availability derived from root exudates and other biological processes associated with frequently irrigated soil are likely to be higher. Poor availability of CH_4 and high concentration of O_2 have been show to have little effect on the community of Type I methanotrophs. This has been demonstrated using soil-borne Type I and II methanotrophs in a laboratory generated gradient of partial pressures of both CH_4 and O_2. In the lowest concentrations of CH_4 and highest concentrations of O_2 colonies were predominantly from Type I, where as at the highest concentrations of CH_4 and lowest concentrations of O_2 colonies were predominantly from Type II (Amaral *et al.* 1995). Following on from this, other workers have shown a negatively declining relationship with depth of Type I methanotrophs in meadow soils (Horz *et al.* 2002). A relationship can be described between NH_4^+ and methanotroph activity, with a less active community where there is a N shortage (de Visscher and van Cleemput 2003). The greens receive the highest nitrogen addition of all areas on a golf course, therefore it is unlikely that NH_4^+ concentration would become a limiting factor. Results may be significantly different for surfaces constructed to USGA specifications, the greens of this golf course are standard push up constructions (Table 2.3). USGA rootzone materials have considerably lower values for CEC therefore nutrients such as NH_4^+ will not be retained to the same extent.

Tees: The biomarker 18:1ω9 t and 18:1ω9 c dominates the community found on the tee. These biomarkers are indicative of (Gram negative) bacteria. The development of a bacterially dominated community on this surface can be related to the process of construction. The tees of this golf course were made by consolidating the soil bulk density compared to the fairway, so they are capable of taking a greater amount of foot traffic. Greater management controls are implemented to select species of grass which grow in this area. As such the tee can be considered as a small area of improved grassland, surrounded by an area of less improved grassland (the fairway). Work carried out in a range of different management intensity grasslands has shown that soil microbial community structure of improved grassland is dominated by bacteria, particularly Gram negative strains (Grayston *et al.* 2004).

Fairways and Roughs: The microbial community structures found on these two areas of the golf course were not significantly different (Figure 7.5a), indicating a community rich in anerobic sulphur reducing bacteria (cyc19:0). The mechanism causing this difference in microbial community can be related to both golf course management and construction. The fairway and the rough receive the smallest amount of resources per unit area. This means there are fewer controls over grass and weed species in these areas and little or no time and money is spent on draining these areas. Anaerobic conditions can therefore develop rapidly, especially at the time of sampling (March 2006) when precipitation had been high in the preceding autumn and winter.

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Chapter 8: The casting behaviour and vertical distribution of the anecic earthworm *Lumbricus terrestris* in different golf course surfaces determined using laboratory microcosms.

8. 1. Introduction

The fundamental processes that govern the vertical distribution of earthworms within the highly engineered soil profiles of a golf course, and how this relates to surface cast formation, are pertinent to developing potential control methods for earthworms. Present knowledge suggests that USGA specification greens represent a hostile environment for earthworms since the rootzone consists of 80-90 % sand. Experiments have shown that earthworms have a preference for soils with a higher clay content (Baker *et al.* 1998) and are less prevalent at extremes of pH (Lee 1985; Sims and Gerard 1999). These assumptions, based on known earthworm behavioural ecology, have provided direction for previous research in control mechanisms of earthworm casting on golf courses without the use of chemicals. Two methods have been predominantly trialled: top dressing with angular sands or other abrasive materials (Williamson and Hong 2005) and the acidification of the soil using sulphate based fertilisers (Baker *et al.* 1996). Both of these trials failed to provide an effective non-chemical control mechanism for earthworms on golf courses when compared to contemporarily available vermicides.

Generally, the activity of earthworms in high sand content soils is limited (Pilar Ruiz *et al.* 2006), however earthworm cast surveys, carried out in Chapter 3 showed that casts, and therefore earthworms, were, or may be still present where USGA rootzone is used in constructions. To elucidate the effects of construction materials and methods for soil profiles of golf courses on earthworm behavioural ecology, an experiment was designed

using microcosms under artificial lights in the experimental glass houses of Cranfield University at Silsoe. Despite the potential limitations of microcosm experiments through increased stress to earthworms through the imposed spatial constraints, and potential for over simplification of environmental parameters, they remain as one of the principal methods for scientific investigation of earthworm behaviour. In excess of 35 papers have been published using this methodology since 2000 (ScienceDirect.com Staff 2006). The experiment was designed to test the hypotheses:

Hypothesis 8.1. The construction profile of a playing surface on a golf course will affect the surface casting characteristics of the anecic earthworm *Lumbricus terrestris*, due to differing construction techniques and materials used.

Hypothesis 8.2. The construction profile of a playing surface on a golf course will affect the vertical distribution of the anecic earthworm *Lumbricus terrestris* through that profile due to differing construction techniques and materials used.

8. 2. Materials and Methods

8. 2. 1. Microcosm design

Wooden microcosms, 520 x 350 x 100 mm (H x W x D) were constructed in order to make assessments of where in the construction profile earthworms were making their burrows (Figure 1.4). Microcosms were constructed so that one dorsal side could be removed to enable observations of the vertical soil profile.

Five treatments, with four replicates, were prescribed to represent the most diverse construction profiles found on the five golf courses used throughout these studies (see Chapter 2.8). These were as follows:

- USGA specification green (designated UG)
- Standard green (designated G)
- USGA topped tee (designated UT)
- Standard tee (designated T)
- Fairway (designated F)

Each treatment profile was constructed following the different specifications for that surface (Figure 8.1). The bulk densities of soil in the microcosms were varied by treatment to simulate the different amount of golf playing traffic and compaction each play surface (treatment) would receive (Balogh and Walker 1992). Parent soil, representing unmodified golf course soil, was collected from Cranfield University at Silsoe Experimental Farm and was classified as a sandy loam (72% sand, 16% silt, 12% clay; 6.1% organic matter), using the Boyocous method (see Chapter 2.4). USGA rootzone (95% sand, 5% silt and clay; 1.6% organic matter) and gravel was acquired from Bailey's of Norfolk Ltd (Norfolk, UK). All treatments were sown with an 80:20 mixture of *Festuca* spp :*Agrostis* spp grass seed (Boughton Loam, Kettering, UK). All treatments were assessed for irrigation requirements every two days and watered on an 'as and when needed' basis.

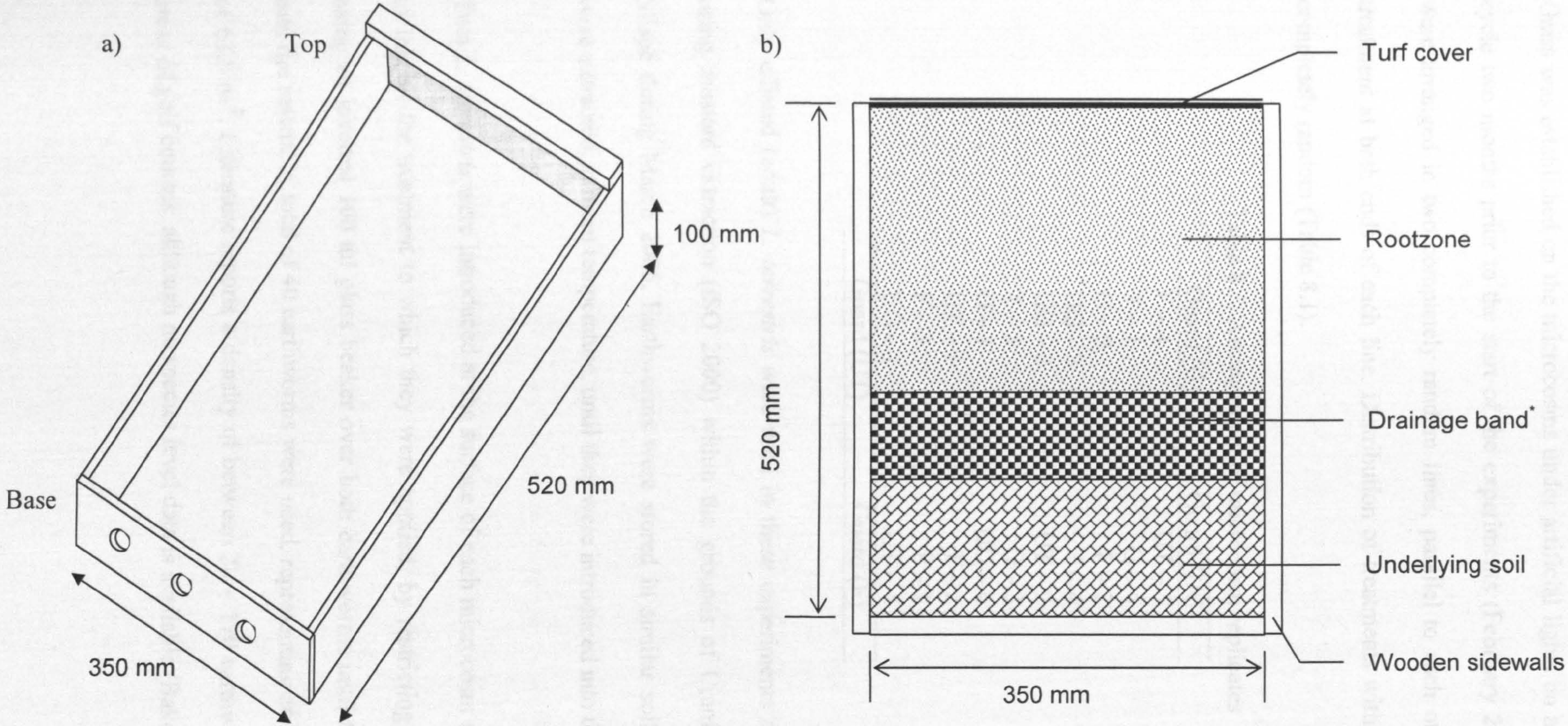


Figure 8.1: Schematic diagrams of microcosms. a) 3D projection of empty microcosm; b) Stylised diagram of filled microcosm (USGA green treatment). Not to scale. * Gravel drainage band not used in all construction profiles

Grass was established on the microcosms under artificial lights on a 16:8 h, light:dark cycle two months prior to the start of the experiments (February 2005). Microcosms were arranged in two completely random lines, parallel to each other, with a guard treatment at both ends of each line. Distribution of treatments within this matrix was completely random (Table 8.1).

Table 8.1: Arrangement of microcosm replicates

Guard (G)	Guard (UG)
F – 4	UT – 3
T – 1	G – 4
G – 2	F – 2
UG – 3	T – 4
UG – 2	UT – 2
T – 3	G – 3
G – 1	UG – 4
UT – 1	T – 2
F – 3	F – 1
UG – 1	UT – 4
Guard (UT)	**Guard (F)**

Only ciliated (adult) *L. terrestris* were used in these experiments and were collected using mustard extraction (ISO 2000) within the grounds of Cranfield University at Silsoe during March 2005. Earthworms were stored in similar soil from which they were extracted, at room temperature, until they were introduced into the microcosms.

Two *L. terrestris* were introduced at the surface of each microcosm and initially forced to inhabit the treatment to which they were applied, by restricting surface migration using an inverted 100 ml glass beaker over both earthworms until they had burrowed into the matrix. A total of 40 earthworms were used, representing an earthworm density of 63.5 m^{-2}. Literature reports a density of between 20 – 170 earthworms m^{-2} on some areas of golf courses, although no species level data is available (Baker and Binns 1998).

After the initial introduction no further constraints were imposed on the earthworms; they were free to migrate to any other microcosm or to completely leave the experimental system.

The grass on each treatment was cut twice per week, with treatments maintained at a different heights to represent the typical different grass clipping inputs to the soil on different play surfaces. The green treatments (G and UG) were cut to a mean height of 5 mm; the tee treatments (T and UT) were cut to a mean height of 10 mm; and the fairway treatments were cut to a mean height of 20 mm. All clippings were left on the surface of the respective microcosms.

8. 2. 2. Data collection and analysis

Prior to the twice-weekly cutting and watering regime, the number of surface casts on each microcosm was recorded. This was carried out for nine weeks (n = 18 per microcosm). Once casts had been recorded they were swept from the surface and cutting and watering was carried out. Analysis of significant differences between treatments was made with generalized linear models (GZM), confidence intervals (± 95%) were used to determine specific treatment differences. After nine weeks microcosms the dorsal side openings were removed, photographed and then hand sorted in 130 mm depth bands to determine earthworm vertical distribution. Differences in distribution were discriminated using multiple analysis of variance (MANOVA). Soil samples were taken from each depth band and homogenised in the laboratory prior to quantification of microbial biomass carbon, using fumigation extraction (see Chapter 2.6). All data analysis was carried out using Statistica 7.1 (Statsoft Inc. 2005).

8. 3. Results

8. 3. 1. Earthworm cast distribution

The formation of earthworm casts was a statistically infrequent event, with 75% of all readings taken over the nine week experiment being zero. The data followed a Poisson distribution (proportion of linear covariance $r^2 = 0.99$ using VassarStats (Lowry 2005)). Analysis using GZM with a log-link function and a Poisson distribution was used to detect significant difference between treatments; $p < 0.01$ (Table 8.2).

Table 8.2: Mean casts per observation on each treatment (n = 72) during the 9 week experiment. Letters show homogenous groups determined using 95% confidence intervals

Treatment	Mean casts per microcosm observation	SE	Confidence Interval	
			- 95%	+ 95%
Standard green	0.25 b	0.06	0.14	0.36
USGA green	0.82 c	0.14	0.55	1.09
Fairway	0.08 a	0.04	0.01	0.16
USGA topped tee	0.50 b,c	0.10	0.31	0.69
Standard tee	0.31 b	0.09	0.13	0.48

Two distinct groups exist in this data, USGA greens and USGA topped tees had the greatest earthworm activity associated with them (mean earthworm casts per microcosm). The fairway treatment had the least casting associated with it of all the surfaces tested, a 10-fold reduction compared to the USGA greens. Treatments representing standard greens and standard tees were not significantly different from each other and intermediate to the differences between the fairways and treatments that include USGA rootzone in their construction.

Significant differences in the size of the soil microbial community of different treatments were also recorded ($p < 0.01$; Table 8.3). Treatments constructed with only sandy loam soil (fairways, standard tees and standard greens) supported almost twice

the soil microbial biomass of those with USGA rootzone in their construction (USGA greens and USGA topped tees).

Table 8.3: Mean soil microbial biomass C from each treatment (n = 4). Letters indicate homogenous groups, assigned using Fisher LSD.

Treatment	Mean microbial biomass C ($\mu g\ g^{-1}$)	SE
Standard green	356 b	8.2
USGA green	183 a	19.5
Fairway	374 b	14.7
USGA tee	173 a	25.7
Standard tee	331 b	15.2

A correlation between earthworm cast frequency and soil microbial biomass carbon was apparent (Figure 8.2). This indicates that a smaller microbial community in the soil is associated with an increase in earthworm casting.

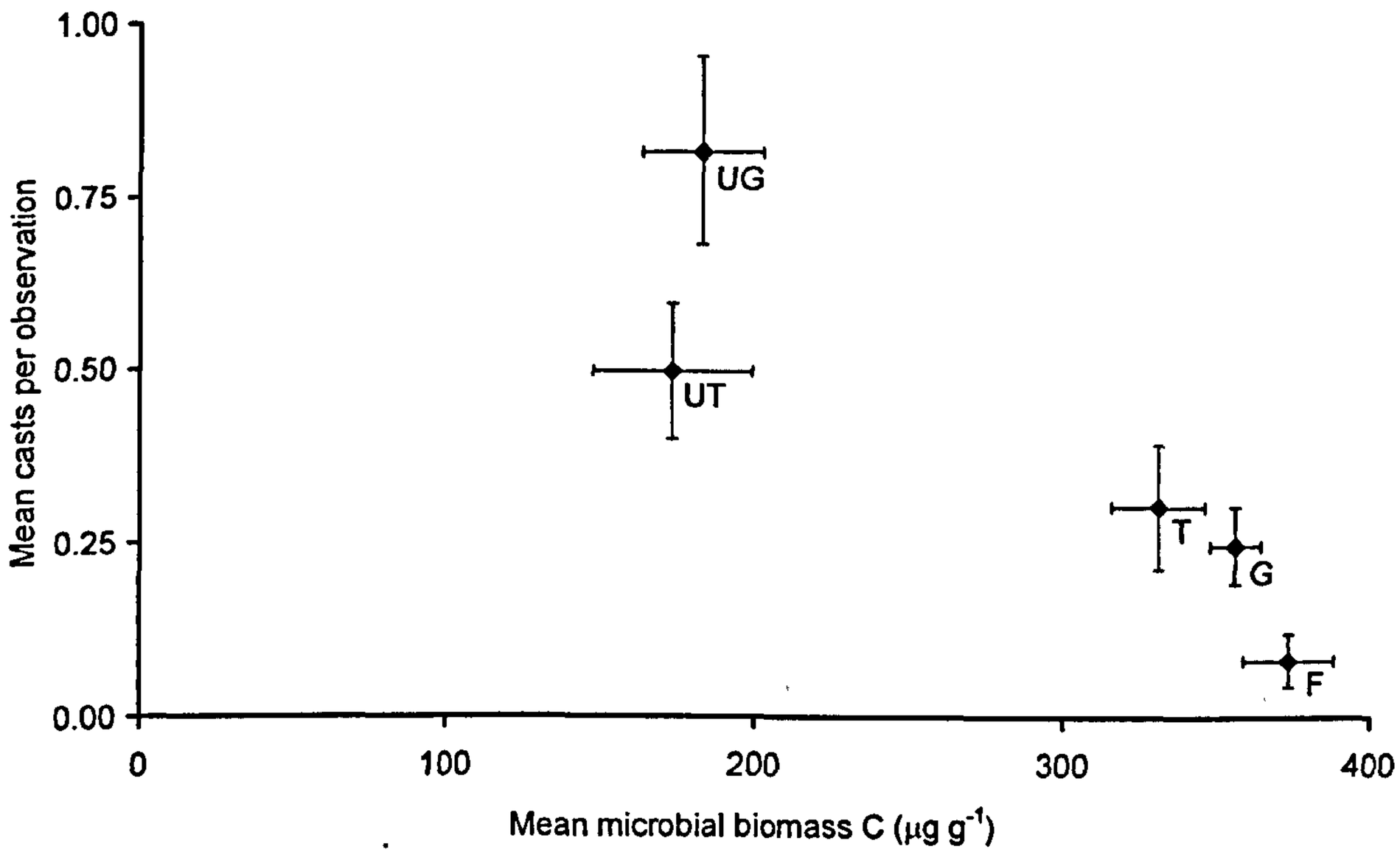


Figure 8.2: The relationship between mean earthworm cast formation per treatment (n = 72) and mean soil microbial biomass C (n = 4) for standard green (G); USGA green (UG); fairway (F); standard tee (T) and USGA topped tee (UT).

8. 3. 2. Vertical earthworm distribution

A total of 40 *L. terrestris* were introduced to the microcosms, of which 39 were recovered at the end of the nine week experiment. The total number of earthworms in each microcosm did not significantly differ by treatment at the end of the experiment ($p = 0.468$). At the time of sampling no earthworms were found below 390 mm. Despite this, evidence of activity below this level was observed (Figure 8.4a-e).

MANOVA of earthworms recovered by handsorting of different depth bands showed that both treatments and depths were significantly different ($p = 0.01$). Residuals from the analysis were normally distributed at all depths despite the small number of observations. There were several interactions within the data which are interpreted using post-hoc Fisher LSD (Figure 8.3).

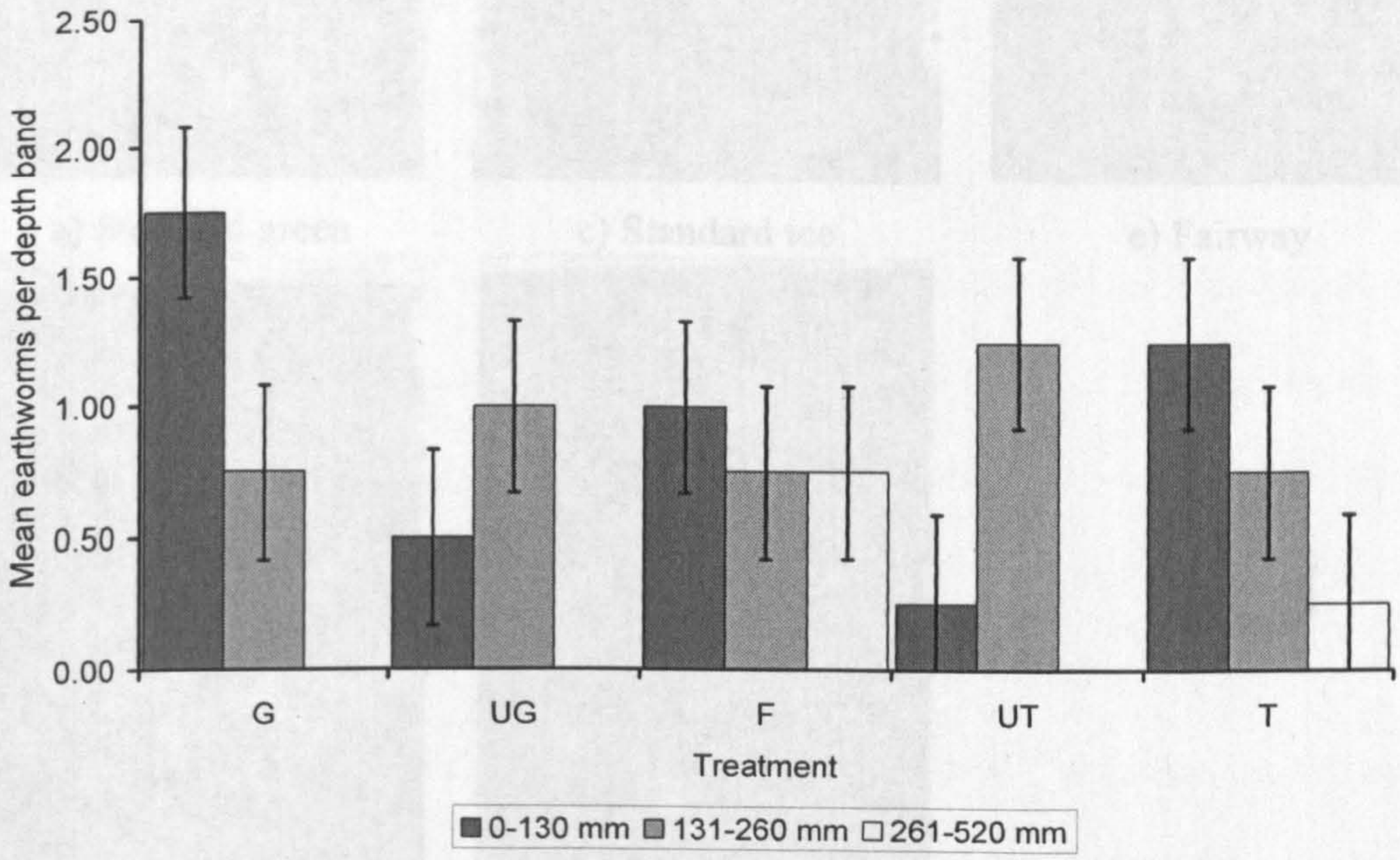
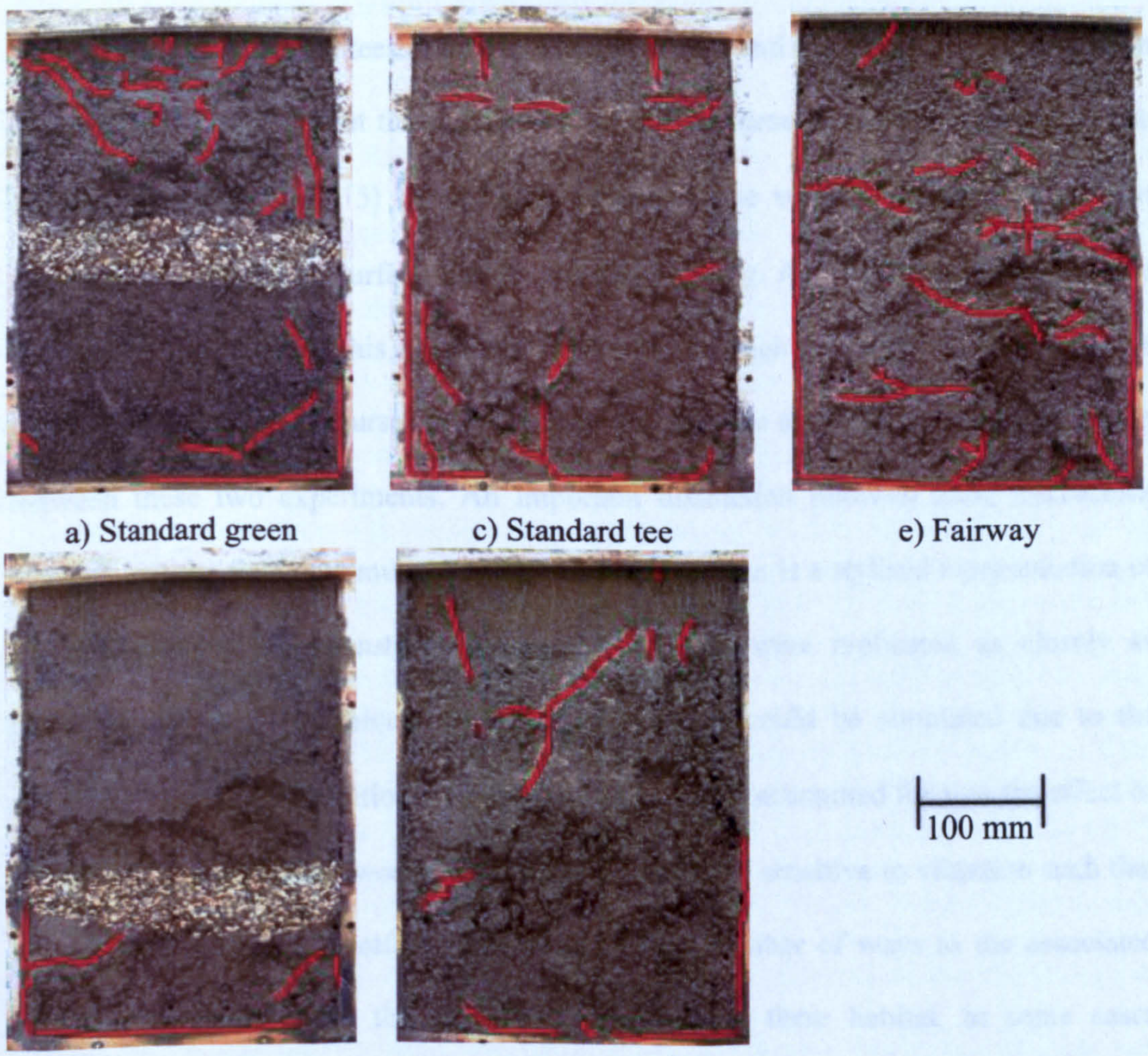


Figure 8.3: Mean earthworms recovered by handsorting microcosms in 130 mm depth bands below the surface (n = 4 per treatment). Whiskers show pooled standard error.

In the fairway treatment there were no significant differences in number of earthworms recovered at any of the depths. The standard green treatment had significantly more earthworms in the 0 – 130 mm depth band than at subsequent depths, a trend that was also recorded in the standard tee treatments. The lowest population of earthworms was recorded in the shallowest depth band of the USGA topped tee treatment with significantly more being found deeper in this soil profile. This trend can also seen in the USGA greens where earthworms tended to be found deeper in the soil profile (Figure 8.4b).



a) Standard green

c) Standard tee

e) Fairway

b) USGA green

d) USGA tee

Figure 8.4a-e: Photographs showing typical vertical sections through differently constructed profiles, selected at random from each treatment. Red lines identify and highlight earthworm burrows visually imposed onto photographs.

8. 4. Discussion

These data did not show the same trends in earthworm casting activity as recorded for annual trends on golf courses in Chapter 3 (*viz.* a ranking of fairways > tees > greens). Broadly these experiments ranked as greens > tees > fairways. It was not possible to ensure that a comparable size of the earthworm population causing the casts on the surface is present in both investigations. Mustard extraction from the fairways of golf courses showed a wide range of earthworm densities on fairways, some of which were comparable to the density used in this trial (recorded earthworm densities of fairways, Max = 34 m^{-2}, Min = 3 m^{-2}, see Table 5.2). It was not possible to make surveys of earthworm density on the tees or greens of golf courses and the difference in earthworm cast frequency implies that the population density on these surfaces is different to the fairway (Chapters 3 and 5) being made between these verify the anecic earthworm population size on each surface used in the field survey. A *L. terrestris* population of 63.5 per m^2, as used in this experiment, may be too high to accurately represent the greens and tees of golf courses, hence it was not possible to ensure a valid comparison between these two experiments. An important distinction between these microcosm systems and the field data must be made: each microcosm is a stylised representation of the environment. The construction and maintenance were replicated as closely as possible but not all techniques used on golf courses could be simulated due to the reduction in scale. An additional factor that could not be accounted for was the effect of people playing golf. Earthworms have been shown to be sensitive to vibration such that is generated by human footfall. They respond in a number of ways to the associated burrow degradation from the physical disturbance to there habitat, in some cases potentially burrowing deeper and in others leaving the soil profile completely (see

Chapter 5). Despite the potential limitations these experiments showed extremely useful trends.

8. 4. 1. Earthworm cast frequency

Data analysis using GZM showed that Hypothesis 8.1 is accepted; $p < 0.01$ (*The construction profile of a play surface on a golf course will affect the surface casting characteristics of the anecic earthworm* L. terrestris *due to differing construction techniques and materials used*). The mechanisms proposed in Chapter 3 to explain significant differences in earthworm casting on different surfaces of golf courses are also applicable to account for the differences recorded here.

Treatments which include the greatest proportion of sand in the construction are more susceptible to having earthworm casts at an unacceptable density to green-keepers and golf players. All anecic earthworms maintain permanent burrows and when they are formed in soils with a high sand content (e.g. USGA rootzone) these burrows must be evacuated more regularly to maintain their stability, hence a greater frequency of casting. Earthworm burrows require more maintenance in this soil matrix due to the lower energy required to overcome the adhesive and cohesive forces between the soil matrix particles (Brady and Weil 1999). These bonds are broken with greater ease than the bonds between soils with lower sand contents; therefore the physical structure of the burrow is damaged more frequently. Evacuated materials (casts) are also more likely to be broken down on the surface, and then fall back into the burrow. Frequent disturbance, such as the twice weekly watering regime may have provided sufficient energy to damage earthworm burrow structures. This in turn results in a significantly higher rate of cast formation.

A reduction in casting in standard tee, standard green and fairway treatments, compared to the USGA green and USGA topped tee treatments are more likely to have been caused by an increase in directly available food from within the soil matrix. *L. terrestris* are incapable of feeding directly from plant material as they lack the appropriate digestive enzymes (Lee 1985). They therefore must rely on the soil biota for their food production and while are frequently cited as being more active in soils rich in organic matter have been shown to feed on soil microbial biomass and its metabolites rather than directly on this organic matter (Lee 1985; Martin *et al.* 1992). The USGA rootzone supports a smaller microbial community than the other soils used in this experiment (Table 8.3) a factor that can be associated with the low soil organic matter content (Kaiser *et al.* 1992). An individual *L. terrestris* specimen in a treatment containing USGA rootzone must ingest (and therefore egest) more soil to maintain the same calorific intake as an individual in a treatment that does not use USGA rootzone in its construction. This therefore results in a higher frequency of casting. Similar effects have been recorded in microcosms containing another earthworm of the family Lumbricidae (*Dendrobaena octaedra*). When earthworms were presented with decreasing quality foods, i.e. those from which it was hard for earthworms to extract their calorific requirements, surface foraging was reduced, however cast formation increased as earthworms increased their soil ingestion rates (Flegel and Schrader 2000).

Many golf course managers consider that earthworms will not prosper when a high proportion of sand is present (*personal communications*). The absence of a significant difference between the standard tee and USGA tee treatments ($p > 0.05$) suggests that the golf industry's assumption with regards to earthworms in this soil type is incorrect.

The rejection of this idea is further reinforced when the USGA green treatment is considered. There was a significant difference between the number of earthworm casts formed on this treatment compared to the standard green treatments ($p < 0.05$), with USGA greens having the greatest casting rate per microcosm. This treatment was constructed with USGA rootzone to a depth of 350 mm from the surface, based on the hypothesis of Williamson and Hong (2005) that no earthworms should be capable of surviving in this ostensibly hostile soil matrix. These differences in findings may have arisen for a number of reasons:

1. The tolerance of UK anecic earthworms (*L. terrestris*) species to hostile soil conditions is (significantly) different from similar species found in the USA (i.e. as investigated by Williamson and Hong, (2005)).
2. The surface migration of the earthworms in this experiment was not constrained, however, the initial act of forcing earthworms to occupy specific microcosms may mean that earthworms formed burrows in soils that they would not usually chose if they were allowed a free range.
3. The earthworm community of the microcosms were unrepresentative of the soil systems that are found on golf courses as only a single species was used.

These potential limitations were considered when the experiments were designed, but were mitigated as far as possible through the experimental design.

Due to the nature of the different construction methods of golf courses, there are only two treatments that can be directly compared, *viz.* standard tees and fairways (Figure 8.1). These were the only treatments that are exclusively constructed from the same soil material. There was a significant reduction in casting between the fairways and the

standard tee, with fairways having the lower value ($p < 0.05$). The principal difference between these treatments was the maintenance regime and the original bulk density to which the treatments were packed. The microcosms representing standard tees were cut to a shorter length than those representing fairways (10 and 20 mm respectively). Data obtained from this experiment was insufficient to test a hypothesis of earthworm control techniques relating to maintenance, such as those suggested by Baker *et al.* (2000). The conclusion of their experiments was that removing grass clippings from experimental plots could reduce the density of earthworm casts. They also noted that this technique had a deleterious effect on turf grass quality. The data from these two microcosm treatments follow similar trends. Fewer earthworm casts were recorded when less grass material was returned to the surface after cutting (fairway treatment). These experiments were not suitable for direct comparison as this treatment effect may have been caused by the different bulk densities of the soil. Research in arable based systems has shown an increase in earthworm cast frequency and density where soil compaction is greatest in comparison to surrounding areas (Binet and Le Bayon 1999). This trend has also been reported between different golf course fairways where increasing numbers of casts were related to increased bulk density (Binns *et al.* 1999).

8. 4. 2. Vertical distribution of earthworms

MANOVA of the data from hand sorting of treatments in 130 mm depth bands means that Hypothesis 8.2 was accepted; $p < 0.01$ (*The construction profile of a play surface on a golf course will affect the vertical distribution of the anecic earthworm* L. terrestris *through that profile due to differing construction techniques and materials used*). Care must be taken when drawing conclusions from this data since the experiment was not replicated temporally.

The fairway treatment represents the most un-amended soil, and consequently there was no difference in the vertical distribution of earthworms (Figure 8.4). The range of earthworm movement through the soil profile can also be seen in Figure 8.4e, with the burrow network being distributed across the whole profile. This is characteristic of the species *L. terrestris*, and has been shown previously using X-ray tomography of repacked soil cores (Bastardie *et al.* 2003). The visual diversity in earthworm burrows in Figures 8.4a-e can be extended to the differences highlighted by Figure 8.3.

Experiments reported in Chapter 7.4 showed that the size of the microbial community (and therefore likely availability of food for earthworms) was different at different depths through the soil profile on golf courses. Other workers have shown similar relationships with the microbial community and depth in other soils (Fierer *et al.* 2003). Therefore where the soil matrix, through its construction specification, does not influence earthworm migration or distribution (e.g. fairway, standard tee and standard green treatments) the greatest proportion of individuals were found at the shallowest depths where food supply was most plentiful (Figure 8.4).

As described in Section 8.4.1, construction profiles with high sand contents did not significantly reduce the frequency of earthworm cast formation. USGA rootzone certainly influences the vertical distribution of *L. terrestris* (Figures 8.3 and 8.4). These findings were similar to those which have been reported for differences in soil particle size distributions (Lee 1985). In the USGA topped tee treatment there was a five fold difference in the number of earthworms recovered from the 0-130 mm depth band (where the USGA rootzone was used in this particular construction) and the 131-260

mm depth band. This vertical distribution was considerably different to that found in the standard tee (Figures 8.4c and d). Laboratory experiments with the anecic earthworm *Hormogaster elisea* (predominantly found in sandy Spanish soils) showed this species negatively selecting soil environments containing course sands as part of the soil matrix. This material was similar in particle size distribution to the USGA rootzone material used in the USGA topped tee treatments. *H. elisea* also positively selected soil environments with higher organic matter content (Pilar Ruiz *et al.* 2006). A similar trend was seen in the USGA green treatments with earthworms predominantly being recovered from deeper in the microcosm where the parent soil was richer in organic matter. Figure 8.4b also indicates that earthworms were predominantly active below the gravel drainage band in the sandy loam soil rather than the USGA rootzone. It is important to note that Figure 8.4 represents only one slice through the each microcosm and that more or less expansive networks could exist behind that slice. These relationships to earthworm burrow structural dynamics can only be fully examined by use of computer aided X-ray tomography scanning similar to the research carried out by Schrader *et al.* (2006).

Chapter 9: The potential mitigation of *Lumbricus terrestris* surface casting behaviour through novel environmental engineering solutions

9. 1. Introduction

As outlined in Chapter 8, the majority of research investigating environmentally benign earthworm control methods has been directed by assumptions relating to earthworm behavioural ecology. These methods have been specifically in relation to soil pH adjustment (Kirby and Baker 1995; Baker et al. 1996), or the use of angular sand particles for top dressing (Williamson and Hong 2005). The most prevalent species identified as a result of the surveys described in Chapter 5, indicate that the combined pH range of these earthworm species is likely to be too wide for the former approach to be successful. The findings of Chapter 8 indicate that *Lumbricus terrestris* is capable of inhabiting soils that are frequently described as being hostile to this species, when they are forced to do so, undermining the latter approach. This research also indicated that where the sand content of the soil is very high such as a USGA specification green, earthworm casting behaviour is increased. Consequently, control of earthworm casting at an acceptable level to the golf industry is unlikely to be achieved with either of these methods.

Two novel approaches were trialled as pilot studies in microcosms. The hypotheses tested were developed as a result of the findings in Chapters 3-7 two environmental engineering solutions were investigated: physical exclusion using a membrane barrier in the soil profile and a soil microbiological control.

9. 2. Materials and methods

9. 2. 1. Experimental design

The microcosms used for these experiments were identical in design to those described in Chapter 8.2. Both trials were carried out in the experimental greenhouses of Cranfield University at Silsoe during autumn 2005 for the physical exclusion experiments and spring 2006 for the soil microbiological control. On all treatments turf grass was established (80:20 *Festuca* spp: *Agrostis* spp grass seed [Boughton Loam, Kettering, UK]) under artificial lights on 16:8 h light and dark cycles. Parent soil, representing unmodified golf course soil, was collected from Cranfield University at Silsoe Experimental Farm and was classified as a sandy loam (72% sand, 16% silt, 12% clay; 6.1% organic matter), using the Boyocous method (see Chapter 2.4). USGA rootzone (95% sand, 5% silt and clay; 1.6% organic matter) and gravel were acquired from Bailey's of Norfolk Ltd, Norfolk, UK. Once turf grass was established, watering was carried out on an as-and-when needed basis. A single species of earthworm (*L. terrestris*) was used throughout these experiments, collected using mustard extraction within the grounds of Cranfield University at Silsoe (ISO 2000). *L. terrestris* was selected due to the findings of earthworm species surveys conducted by Binns *et al.* (1999) and because individuals of this species are large, and hence produce large, easily observed earthworm casts.

Both investigations focused on the ability of earthworms to colonise a newly constructed golf course soil profile. All earthworms were therefore introduced to their respective treatment at the surface. Each earthworm was also forced to initially inhabit its designated treatment by constraining surface migration by confining the earthworms within the bounds of an inverted 100 ml beaker. Once earthworms had formed their

initial burrow they were free to migrate between microcosms or to completely leave the system.

Not all play surfaces found on golf courses were trialled in relation to physical exclusion methods of earthworm cast control. Only the tee area was selected for a number of reasons:

1. The tee is an aesthetically sensitive area of a golf course, and prone to significant earthworm cast activity (see Chapter 3). A successful earthworm control mechanism involving a physical exclusion barrier in this surface would have the lowest impact on course management, with regards to destructive and invasive cultivation practices and still result in the benefits relating to earthworm cast elimination. Physical exclusion of earthworms would also represent an inexpensive solution most practicably realised on the tee area of a golf course.
2. Surveys carried out in Chapter 3, and previous microcosm experiments (see Chapter 8), indicated that when USGA rootzone was used in profile construction, earthworm casting occured at a significantly greater frequency.

A USGA topped tee soil profile, paired with a standard tee construction, makes a particularly appropriate test medium to investigate. The effect of including a physical barrier to the vertical burrowing behaviour of earthworms was tested under the following hypotheses:

Hypothesis 9.1. The inclusion of a physical exclusion barrier at different depths through the construction profile will have a significant impact on earthworm activity,

influencing casting behaviour, resulting in a reduction in earthworm cast formation at the surface.

Hypothesis 9.2. The inclusion of a physical exclusion barrier will affect the vertical distribution of earthworms by limiting their movement to and from the soil surface. The influence on earthworm distribution will be positively correlated to the depth of the barrier in the profile.

Soil microbiological control was trialled as an earthworm casting activity mitigation technique for USGA specification greens. A non-invasive control for earthworms on this surface is therefore highly desirable to the golf industry. Chapter 8 indicated a potential relationship where earthworm cast frequency decreased with an increase in the size of the soil microbial community (Figure 8.3). This relationship was tested further, as a potential means of earthworm cast control under the hypotheses:

Hypothesis 9.3. Increasing the size of the microbial community of the soil will have no effect on the density of *Lumbricus terrestris* individuals in each treatment.

Hypothesis 9.4. Increasing the size of the microbial community in the soil will affect the rate of surface casting of the earthworm *Lumbricus terrestris* by increasing availability to forage (and so therefore casting) within the soil profile.

9. 2. 2. Data collection and statistical analysis

After introduction of earthworms to the microcosms a twice weekly cast survey was carried out, assessing each treatment individually. These observations were made in both experiments and continued for the nine week duration of the experiment (n = 18 per replicate). The distribution of the data was confirmed to be Poisson using VasserStats (Lowry 2005) and treatment differences were discriminated using

generalized linear models (GZM) with post-hoc differences highlighted with ±95% confidence intervals.

In the physical exclusion experiment, differences in vertical distribution of earthworms were analysed using general linear models (GLM) and non parametric statistics. This allowed the determination of differences in earthworm population distributions with regards to pertinent barrier depths (Kolmogorov-Smirnov test). Significant differences in earthworm distribution in the soil microbiological control experiments were analysed using multiple analysis of variance (MANOVA).

9. 2. 3. Physical exclusion

Two different construction methods for tees were investigated in this experiment, a standard construction (denoted S) and a USGA rootzone topped tee (denoted U). A total of 48 microcosms were used, 24 for each profile construction. Three treatments were investigated, with an increasingly deep barrier material included in the construction (Figure 9.1). These were as follows:

- Control, no barrier included in the profile (designated C)
- Barrier included at 25 mm from the surface (designated 25)
- Barrier included at 100 mm from the surface (designated 100)
- Barrier included at 200 mm from the surface (designated 200)

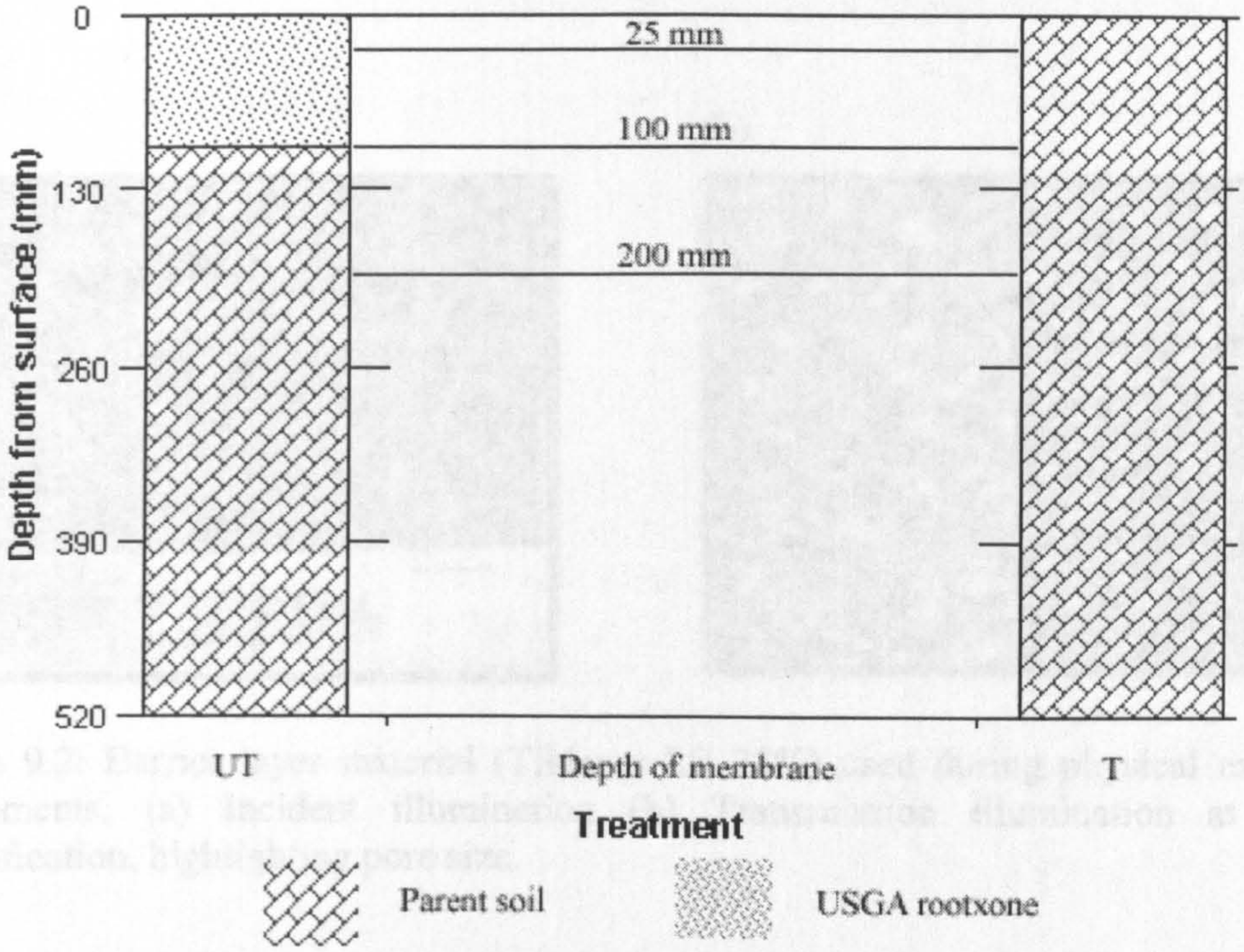


Figure 9.1: Vertical construction of the two different types of tee used in the physical exclusion experiments. Location and depth of barrier layer is indicated in relation to the different soils used in construction.

The material chosen as the physical barrier for these experiments was supplied by Tildenet Ltd. (Bristol, UK). The products intended application is as greenhouse shading, to reduce light transmission by 73% (Figure 9.2). The mean pore opening through this membrane was < 2.5 mm^2, below the average diameter for *L. terrestris*, as reported by Sims and Gerard (1999). Five replicates of each depth of barrier treatment were used per construction profile. The barrier material was continuous to the surface, lining the sides of the microcosms to prevent earthworms burrowing around the barrier material.

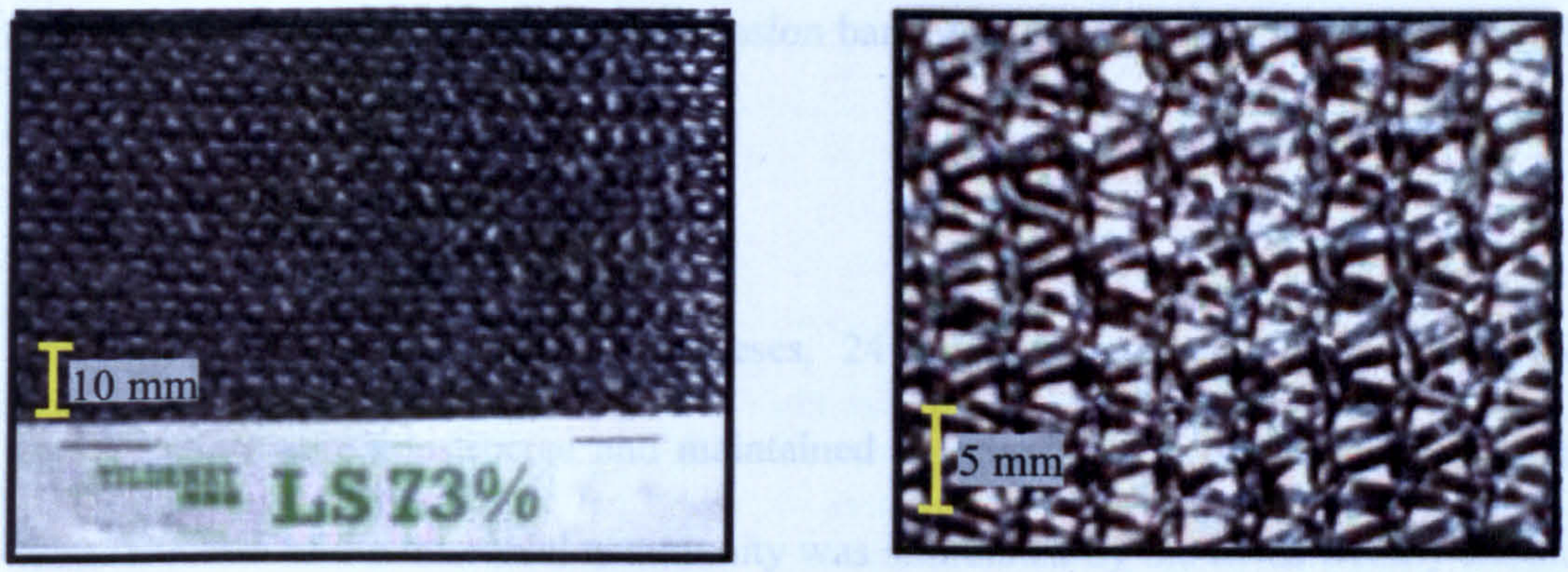


Figure 9.2: Barrier layer material (Tildenet LS 73%) used during physical exclusion experiments. (a) Incident illumination (b) Transmission illumination at higher magnification, highlighting pore size.

All replicates were arranged in a four of parallel lines, two microcosms wide, at random, thus avoiding a statistical blocking effect (Table 9.1). Guard treatments were placed at each end of the lines to ensure that all experimental microcosms experienced the same microclimatic variations. Two adult and one non ciliated juvenile *L. terrestris* were introduced at the surface of each treatment.

Table 9.1: Spatial layout of microcosm treatments for standard tee (S) and USGA topped tee (U) constructions. Number following construction treatment indicates the depth of physical exclusion barrier in millimetres.

Guard (S 25)	Guard (U 200)	Guard (S 200)	Guard (S Control)
U 100	U 200	U Control	S 100
S 100	U 25	U 200	U 25
S 25	S Control	S 200	S Control
S 200	S 25	U 100	S 25
U Control	U 100	U 25	S 100
U 25	S 200	S 100	U 200
S 100	U Control	U 100	S Control
S Control	S 200	U 200	S 200
U 200	U 100	S Control	U 25
S 25	U Control	S 25	U Control
Guard (U 100)	Guard (U Control)	Guard (S 100)	Guard (U 25)

After nine weeks of cast survey observations microcosms were deconstructed through the lateral opening, and hand sorted to recover all earthworms. The sexual maturity and position in relation to the physical exclusion barrier of the earthworms remaining in the microcosms was recorded.

9. 2. 4. Microbiological control

In order to investigate these hypotheses, 24 microcosms using the USGA green specification were constructed and maintained as per this type of surface (see Figure 8.1). The size of the microbial community was stimulated by the twice weekly addition of glucose solution formulated to increase the biomass by increasing magnitude. A gradient of microbial community size was thus established using three different concentrations of glucose. These treatments were:

- Control, no glucose added (denoted Control)
- Targeted two-fold microbial biomass increase (denoted x2)
- Targeted four-fold microbial biomass increase (denoted x4)
- Targeted six-fold microbial biomass increase (denoted x6)

The newly installed USGA greens at Buckingham golf club were considered as most comparable to the greenhouse treatments. Mean microbial biomass C measured on these surfaces eight weeks after they had been completed was 77.5 $\mu g\ g^{-1}$ (SE = 12.2) using chloroform-fumigation extraction (Chapter 2.6). This was used as a baseline population size from which the amount of additional glucose substrate to reach the required microbial population size was estimated (Table 9.2).

Table 9.2: Calculations of additional carbon substrate required to achieve the requisite targeted increase in microbial community size of USGA green microcosm treatments. Details of calculations are outlined in Equations 9.1 – 9.6.

Treatment	Estimated initial Biomass	Target Biomass	Additional Biomass required
(a)	(b)	(c)	(d)
Control (0)	77.5	77.5	0
x2	77.5	155.0	77.5
x4	77.5	310.0	232.5
x6	77.5	465.0	287.5

Treatment	Additional Biomass required	Additional µg C g^{-1} soil	Additional g C required per microcosm	Additional g glucose required per microcosm	Application concentration g L^{-1} at 250 mL per microcosm
	(d)	(e)	(f)	(g)	(h)
Control	0	0	0	0	0
x2	77.5	193.75	3.49	8.73	34.9
x4	232.5	581.25	10.46	26.15	104.6
x6	287.5	968.75	17.44	46.30	174.4

$$c = b \times a \quad (9.1)$$

$$d = c - b \quad (9.2)$$

$$e = \left(d/40\right) \times 100 \quad (9.3)^{8}$$

$$f = \frac{(e \times 18000)}{10^{6}} \quad (9.4)^{9}$$

$$g = \left(f/40\right) \times 100 \quad (9.5)^{10}$$

$$h = g \times \left(1000/25\right) \quad (9.6)$$

Five replicates were used per treatment, arranged in two completely random blocks, parallel to each other, with a guard treatment at both ends (Table 9.3). For the four weeks preceding earthworm introduction, and during the nine weeks of earthworm cast observations, glucose solutions were applied to each treatment. On the initial application of glucose solution, a nitrogen fertiliser (Scotts Preseeder PS5 8:12:8,

[8] Estimated assimilation rate of soil biota was approximately 40% (Degens and Harris 1997).
[9] 18 kg of USGA rootzone was used in the construction of each microcosm.
[10] M_r Glucose ($C_6H_{12}O_6$) = 180 g mol^{-1} of which 40% w/w is carbon.

Suffolk, UK) was also applied at 30 µg mm^{-2} on the control treatment and at two-fold, four-fold and six-fold the control rate in order to maintain a suitable C:N ratio for microbial growth in the soil.

Table 9.3: Layout of microcosms in the soil microbiological control of earthworm casting experiments.

Guard (x6)	Guard (x4)
x6 – 1	x4 – 1
x2 – 1	x2 – 2
x6 – 2	Control – 1
x4 – 2	x6 – 3
x2 – 3	Control – 2
Control – 3	x4 – 3
x6 – 4	x2 – 4
x2 – 5	x4 – 4
x4 – 5	Control – 4
Control – 5	x6 – 5
Guard (C)	Guard (x2)

9. 3. Results

9. 3. 1. Physical exclusion

The behaviour of earthworms introduced to both physical exclusion construction treatments was similar, with 75% of all *L. terrestris* used in the experiment being recovered at the end of the trial. More earthworms migrated from the standard construction tees (27%) compared to the USGA topped tees (21%) but no significant differences were recorded between population sizes in any treatments ($p > 0.05$ in all cases).

Table 9.4: A comparison of the total number of *L. terrestris* individuals introduced at the beginning of the nine week experiment and those remaining at the end with regards to different construction profiles.

	Initial introduced	Migrated from microcosms	Recovered	Treatment			
				C	25	100	200
Standard tee	60	16	44	10	9	13	12
USGA topped tee	60	13	47	10	10	14	13
Total	120	29	91	20	19	27	25

9.3.1(a) Casting behaviour

Frequency analysis of the earthworm cast data recorded during the nine week survey period indicated a Poisson distribution. Observed vs. expected proportion of linear co-variance, $r^2 = 0.999$ (Lowry 2005) – See Appendix V. GZM with a construction x barrier depth interaction indicated a significant difference in earthworm casts per observation on the different construction types ($p = 0.04$). Within the standard tee construction the depth of the barrier had no significant effect on the earthworm cast frequency per observation ($p > 0.05$). The depth of the barrier in the USGA topped tee construction did however, have a significant effect ($p < 0.01$); Figure 9.3. Interpretation of ±95% confidence intervals for this construction treatment showed that the physical exclusion barrier at 200 mm below the surface in the USGA topped tee treatment resulted in a significant reduction in earthworm casts per microcosm observation compared to those at all other depths, including the control treatment.

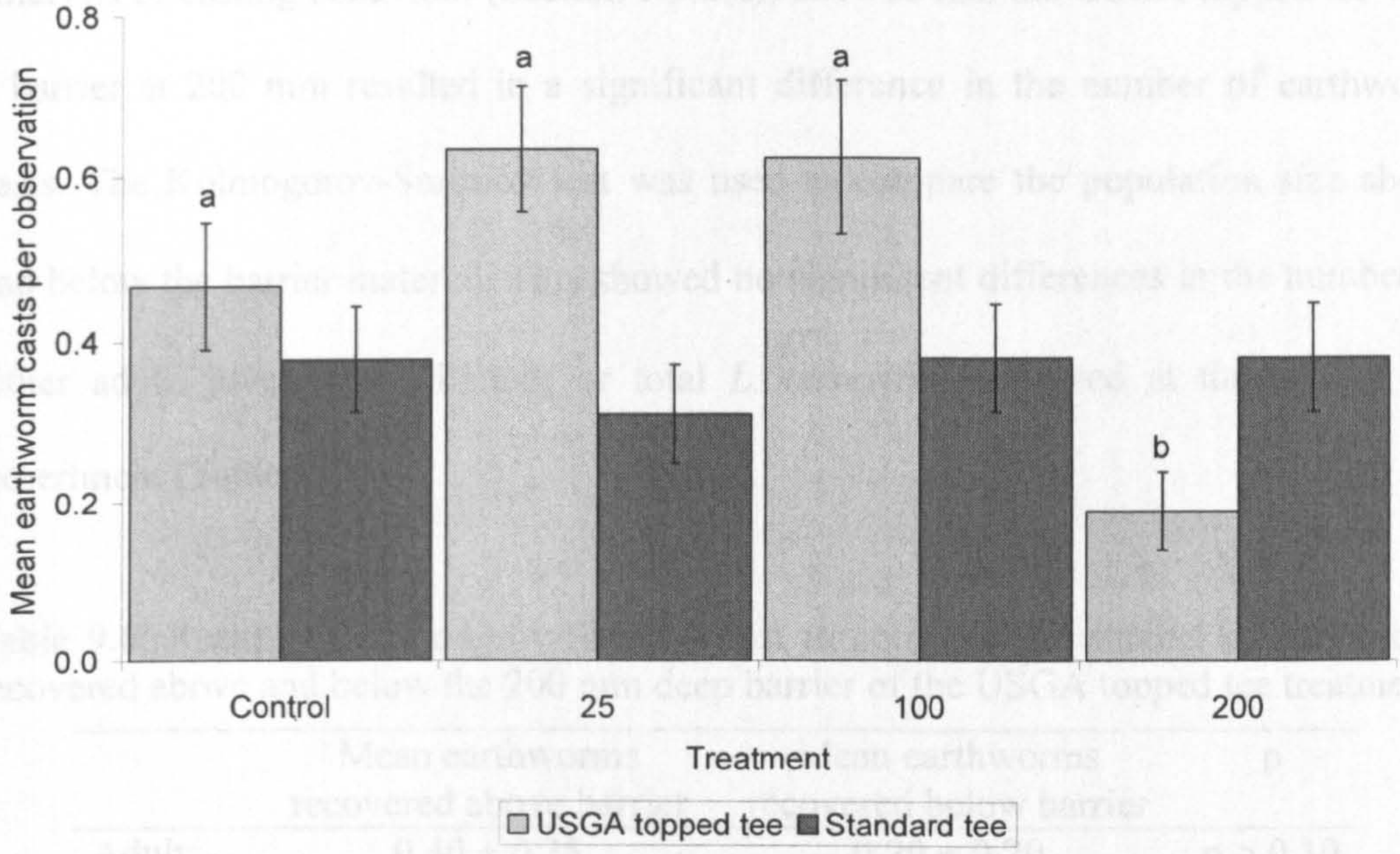


Figure 9.3: Differences in earthworm casting per observation on all microcosms for each treatment (n = 5 per treatment). Whiskers show standard error of the mean, letters indicate homogenous groups determined by ±95% confidence intervals.

9.3.1(b) Vertical distribution

The vertical distribution of the earthworms that remained in the experiment was analysed using a GLM with a construction x barrier depth interaction. This showed no significant differences in the distribution of *L. terrestris* within the microcosms after the nine week experiment in relation to the depth of the physical exclusion barrier (Table 9.5). Despite the small population size (n = 40) the residuals from this analysis showed a normal trend (see Appendix V).

Table 9.5: Analysis of the effect of barrier depth on vertical earthworm distribution from all microcosms after nine weeks of cast surveys (n = 40).

	Degrees of freedom	F	p
Construction	1	0.08	0.78
Barrier depth	3	0.25	0.86
Construction x Barrier depth	3	0.62	0.61
Error	32		

Analysis of casting behaviour (Section 9.3.2(a)) showed that the USGA topped tee with a barrier at 200 mm resulted in a significant difference in the number of earthworm casts. The Kolmogorov-Smirnov test was used to compare the population size above and below the barrier material. This showed no significant differences in the number of either adult, juvenile (unciliated) or total *L. terrestris* recovered at the end of the experiment (Table 9.6).

Table 9.6: Results of Kolmogrov-Smirnov test to compare the number of earthworm recovered above and below the 200 mm deep barrier of the USGA topped tee treatment.

	Mean earthworms recovered above barrier	Mean earthworms recovered below barrier	p
Adults	0.40 ± 0.25	0.20 ± 0.20	$p > 0.10$
Juveniles	1.20 ± 0.58	0.20 ± 0.20	$p > 0.10$
Total	1.60 ± 0.74	0.40 ± 0.40	$p > 0.10$

9. 3. 2. Microbiological control

A total of 40 *L. terrestris* were introduced to microbiological control microcosms at the start of the casting observations, by the end of the nine weeks only 25 remained (Figure 9.4). The other earthworms either emigrated from the experimental system or died within it. The emigration of earthworms from the experiment was independent of treatment; no significant differences were detected in population size of each treatment at the end of the experiment ($p > 0.05$).

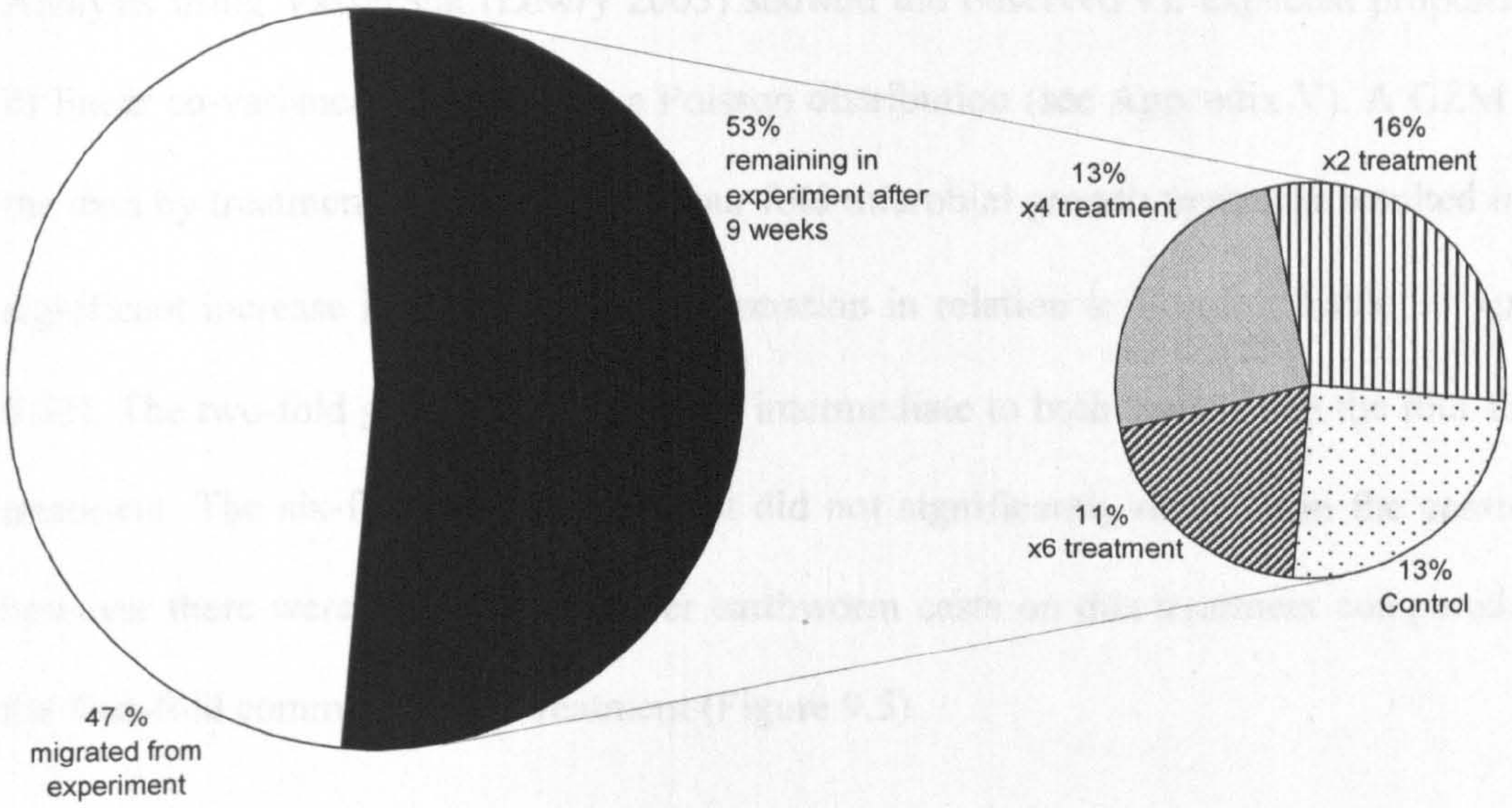


Figure 9.4: Pie charts showing the breakdown of the number of earthworms found in each treatment at the end of the nine weeks, accounting for those that migrated from the experimental system during the trial.

Replicate-by-replicate inspection of the data indicated that some microcosms had no earthworms residing in them at the end of nine weeks. These replicates were excluded from the subsequent analysis of earthworm cast frequency.

9.3.2(a) Vertical distribution

There was no significant difference in either the number of earthworms recovered by handsorting between treatments; Table 9.7 ($p > 0.05$) or any significant difference in their vertical distribution within the treatments established using MANOVA ($p > 0.05$).

Table 9.7: Distribution of earthworms recovered from different treatments in the soil microbial control of earthworm casting experiments.

Treatment	n	Total earthworms recovered per microcosm	SE
Control	5	1.0	0.45
x2	5	1.2	0.58
x4	5	1.0	0.45
x6	5	0.8	0.20

9.3.2(b) Casting behaviour

Analysis using VasserStat (Lowry 2005) showed the observed vs. expected proportion of linear co-variance, $r^2 = 0.96$ for a Poisson distribution (see Appendix V). A GZM of the data by treatment showed that the four fold microbial growth treatment resulted in a significant increase in earthworm cast formation in relation to Control; Table 9.8 ($p = 0.03$). The two-fold growth treatment was intermediate to both control and the four fold treatment. The six-fold growth treatment did not significantly differ from the control, however there were almost 40% fewer earthworm casts on this treatment compared to the four-fold community size treatment (Figure 9.5).

Table 9.8: Generalized linear model of earthworm cast observations on different soil microbial control treatments (treatments containing no earthworms at the end of nine weeks excluded).

Treatment	Observations per treatment	Mean casts per observation	SE	Confidence interval	
				-95 %	+95 %
Control	51	0.18 a	0.05	0.07	0.28
x2	51	0.27 a,b	0.07	0.12	0.42
x4	51	0.33 b	0.08	0.17	0.50
x6	68	0.09 a	0.04	0.01	0.17

ANOVA of the microbial community size on each treatment showed significant differences between treatments ($p < 0.01$) after 13 weeks of glucose solution application. An effective gradient in size of microbial population was established however no treatment reached its target population size. Earthworm cast frequency on each microcosm was compared to the measured size of the microbial population. This highlighted where a low microbial biomass results in a high frequency of earthworm cast formation and tended to decline after a threshold of microbial biomass was reached (Figure 9.5). A highly significant 2nd order polynomial relationship, with the intercept

forced to zero, was evident (r^2 = 0.92) as it is predicted that in a system with no microbial community that all earthworms would either die or emigrate from the system.

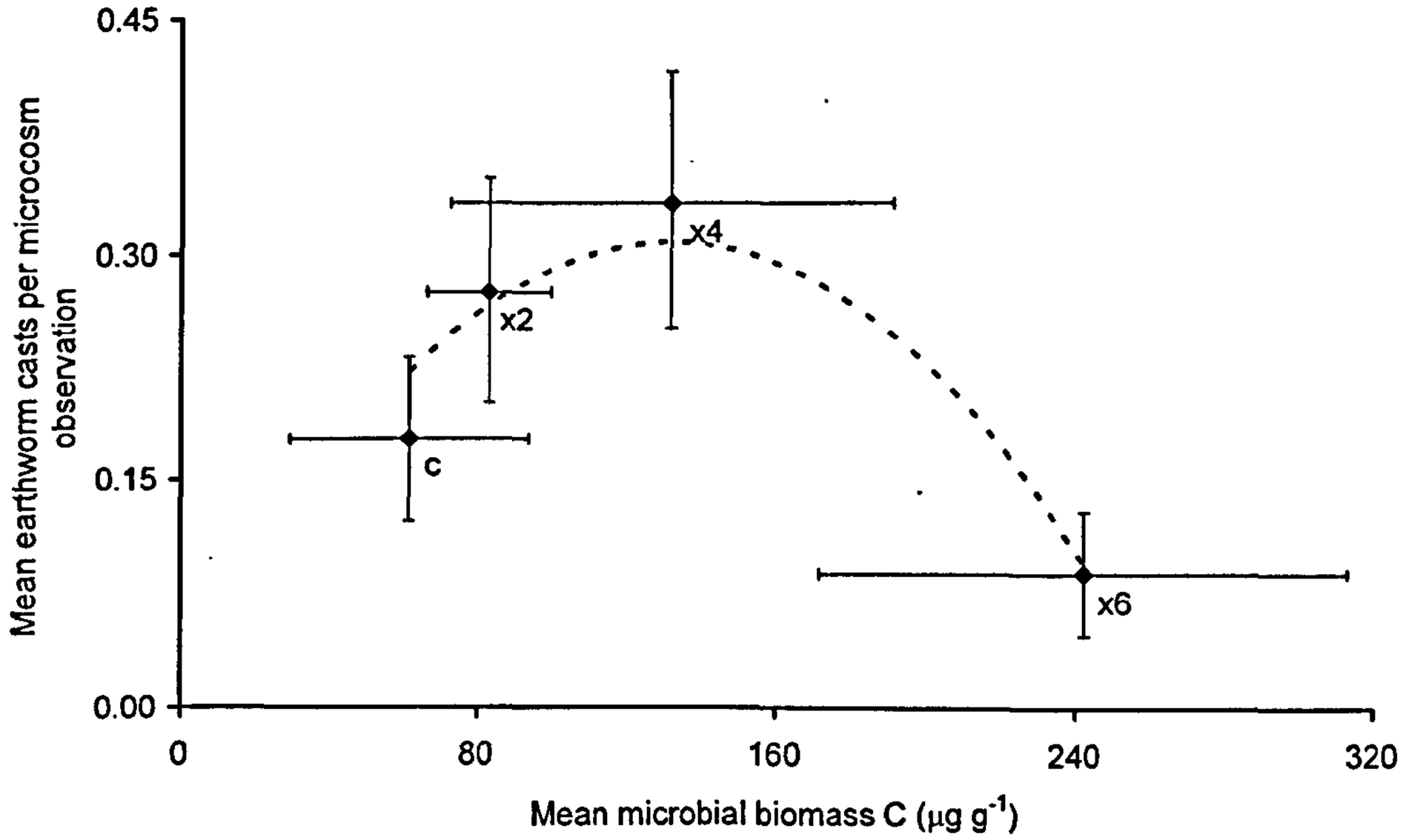


Figure 9.5: Relationship between mean earthworm cast observations and the size of the soil microbial community. Treatments indicated Control (c), and growth stimulations at two (x2), four (x4) and six (x6) fold (attempted) increases. Whiskers show standard error of the mean. 2nd order polynomial relationship (r^2 = 0.92) inferred with a dashed line.

9. 4. Discussion

Both environmental engineering solutions trialled showed potential to be used as a means of controlling the formation of earthworm casts on golf courses. Neither method resulted in a complete eradication of earthworm casts. This is a greater reflection of the adaptive biology of the earthworm than the ineffectiveness of any control method.

9. 4. 1. Physical exclusion

The depths for the barrier were selected because it was considered that the 25 mm deep barrier would represent the most effective control, by preventing any earthworms establishing burrow networks in this treatment. However, practical problems for turf grass husbandry would be presented by this solution. Some aeration techniques, such as

hollow tine cultivations penetrate the soil to depths of up to 200 mm. Hence the treatments at 100 and 200 mm were included to represent potential solutions where some penetrative cultivation techniques could still be carried out.

The mean number of earthworm casts per microcosm data recorded during this experiment for standard tee (0.32; S.E. = 0.02) and USGA topped tee (0.47; S.E. = 0.03) constructions were comparable to the findings relating to equivalent profile constructions used in Chapter 8 (see Table 8.2). This indicated that the microcosm investigation technique is robust, and the findings have a validity that could be scaled up to whole golf course circumstances.

The inclusion of a physical barrier to vertical earthworm movement in different tee constructions showed no effect with regards to the standard tee. Therefore in relation to this construction profile Hypothesis 9.1 (*The inclusion of a physical exclusion barrier at different depths through the construction profile will have a significant impact on earthworm activity, influencing casting behaviour, resulting in a reduction in earthworm cast formation*) must be rejected when using the Tildenet LS 73% material. In the USGA topped tee construction treatments, there were significantly fewer earthworm casts per observation when a barrier was included at a depth of 200 mm. All other treatments were not significantly different to the control. As a result Hypothesis 9.1 is provisionally accepted for the USGA topped tee construction.

Few experiments of this nature have been reported in literature and a causal mechanism for this behaviour is challenging to explain. In the 200 mm deep barrier treatment both USGA rootzone and parent soil were above the barrier. No significant difference with

respect to the number of earthworms recovered from the microcosms was recorded. Therefore a true comparison can be made between each treatment, even though 25% of earthworms introduced migrated from the experimental system. Further analysis of earthworm distribution relative to the barrier using the Kolmogrov-Smirnov test also showed no significant differences in the number of either adults or juvenile earthworms found above or below the barrier material. This same distribution was recorded for treatments with barrier layers at 25 and 100 mm (data not shown). The difference in the number of casts formed in this treatment may be related to habitat selection and feeding preferences regarding the availability of both soil types above the barrier layer. The findings of others, conducting research with *Hormogaster elisae,* indicate that when this anecic earthworm has free choice of its environment it will select nutrient rich soil, with a matrix consisting of a low percentage of sand (Pilar Ruiz et al. 2006). Another suggestion is that earthworms must void their guts in order to pass through the pore space of the barrier, therefore form more surface casts in these treatments. Where earthworm vertical migration is not as constrained with the 200 mm deep barrier, there was less surface casting. This hypothesis may account for the elevated levels of casting in the 25 and 100 mm deep barrier treatments compared to the control (although not statistically significant).

These experiments showed that a physical exclusion barrier with a pore size of 2.5 mm^2 does not significantly alter the distribution of earthworms within this experimental system. This material has some elasticity and so pore space may be flexible, as are the diameter of earthworms. With both techniques of tee construction there was no significant difference in the total number of earthworms recovered per treatment. The

conclusion to this finding must be that the barrier used in this experiment was incapable of physically excluding the earthworms in the experimental system. This was despite a laboratory based trial which indicated that the selected barrier material was capable of inhibiting earthworm movement (data not shown). When earthworms were presented with a sieve modified to hold the Tildenet LS 73% material no adult *L. terrestris* moved through the material, there was some movement through the barrier material by juvenile *L. terrestris*, although no statistical significance was recorded. These experiments provide no evidence to support Hypothesis 9.2 (*The inclusion of a physical exclusion barrier will affect the vertical distribution of earthworms by limiting their movement to and from the soil surface. The influence on distribution will be positively correlated to the depth of the barrier in the profile*) and it is duly rejected.

In the microcosms studied here, the inclusion of a Tildenet LS 73% barrier layer in the USGA topped tee had an effect on the casting behaviour, although all analyses carried out failed to indicate a causal mechanism. Further experimentation is required to elucidate this mechanism. Firstly, it would be pertinent to replicate the experiment to check if the phenomenon recorded here is repeatable (replication in time). In this experiment the standard tee constructions could be eliminated. Once it was firmly established that the inclusion of this barrier does result in a significant reduction in earthworm casts then an investigation of the mechanism causing this difference can be conducted. This investigation could experiment with different pore size materials – the Tildenet material used in these investigations is available in pore sizes from 36 mm^2 to 1.5 mm^2. By using a range of pore sizes it would be possible to determine if it is the presence of the barrier layer or the restrictiveness to migration of this barrier that caused the reduction in earthworm casts. Further investigation of these experimental parameters

using a non-flexible, or ridged barrier layer may also be pertinent. In all of the proposed experiments an increase in the number of replicates would assist the statistical analysis.

9. 4. 2. Microbiological control

During this experiment almost half of the *L. terrestris* initially introduced left the experimental system. Hand sorting the soil profiles in 130 mm depth bands nine weeks after the earthworms had been introduced into the system showed no significant difference in the number of individuals remaining in any treatment ($p > 0.05$). The vertical distribution through the microcosms was likewise not affected by the amount of glucose applied per treatment ($p > 0.05$) therefore Hypothesis 9.3 (*Increasing the size of the microbial community of the soil will have no effect on the number of* Lumbricus terrestris *individuals found in each microcosm*) is accepted.

Earthworm emigration from the experiment can be attributed to two factors:

1. The USGA rootzone is predominantly a sandy matrix (95 % sand). This represents an abrasively hostile environment for the earthworms, even when they had been conditioned for several weeks to live in such an environment. Research with other anecic earthworm species has shown highly discriminatory habitat selection with regards to the particle size distribution of the soil matrix (Pilar Ruiz et al. 2006). These experiments showed earthworms emigrating from microcosm soil treatments that contained a high proportion of sand, in a similar way to the behaviour observed within this experiment. These initial observations of earthworm emigration from the experiment indicate reasoning for attempted control methods through top-dressing with sand as a control method, although subsequent observations in this experiment imply otherwise.

2. The temperature during spring 2006 was unseasonably high (mean max 17.6°C; see Appendix II). The experimental greenhouse reached approximately 10°C warmer than this (no data recorded). Much research has been carried out showing that earthworms are sensitive to this extreme of temperature (Lee 1985; Doube and Brown 1998; Curry 1998; Lowe and Butt 2005; Perreault and Whalen 2006). The water holding capacity of the USGA rootzone is low, due to the large pore spaces between sand particles. Consequentally earthworms that are free to migrate within and out of the system selected to locate new habitats elsewhere to avoid such hostile conditions. This demonstrates that earthworms are sensitive to both soil and environmental conditions. The control of earthworms by manipulating the moisture status of the soil would be untenable on golf courses, inhibiting turf growth.

The *L. terrestris* that remained in the experimental system showed no difference in distribution within treatments. This may have been because once the burrow structure was formed it was not an optimal use of resources for the earthworm to forage across other microcosms and locate treatments with a larger soil microbial community due to potential disadvantages relating to finding a poorer patch. The control treatment represents the lowest calorific value soil to the earthworm, however these experiments indicate that even a microbial biomass C of 61 $\mu g\ g^{-1}$ (SE 31.1 $\mu g\ g^{-1}$) is sufficient to support an individual *L. terrestris*. Further experiments manipulating the nutrient supply to individual earthworms would be required to ascertain what the specific size of the microbial community required to derive sufficient energy to support life.

These experiments support Hypothesis 9.4 (*Increasing the size of the microbial community in the soil will affect the rate of surface casting of the earthworm Lumbricus terrestris by increasing availability to forage (and so therefore casting) within the soil profile*). Where there was postulated to be a greater abundance of earthworm available foods a lower rate of earthworm cast formation was observed. The number of earthworm casts recorded per microcosm observation was taken to be directly related to the amount of foraging that the earthworms resident in that microcosm have done. The higher microbial biomass of the soil represents a higher calorific food resource than foraging for decaying plant matter on the surface of the microcosms. Thus less foraging effort is required to derive an equivalent energy intake where the soil microbial biomass is higher. From this a 2nd order polynomial, hump-back, relationship can be inferred (see Figure 9.5). A similar hump-back relationship is an established ecological phenomenon between feeding intensity and the size of the prey community have been recorded on numerous occasions since the relationship was first noted by Elner and Hughes (1978). Hypothesis 9.4 could be more fully tested by using X-ray CT scanning of intact microcosms to investigate the changes in structure to earthworm burrows in treatments with different sizes of microbial communities. Using such a technique it should be possible to positively identify earthworm casts formed within the soil profile, due to the refractive properties of the associated mucilage and glycoprotein from the earthworm's gut.

The implications for effectiveness are divided into those relevant to new course construction (or rejuvenation) and those for golf course management. For both solutions that have been highlighted, considerable further investigation is required.

Potential control solution trials must be repeated both at the microcosm scale, to ensure the validity of the recorded effects, and then scaled up to larger, outdoor plots.

Chapter 10: Synthesis of research towards the environmentally sustainable mitigation of earthworm cast formation on golf courses.

10. 1. Introduction

Earthworm casts on the surface of golf courses, from the player's perspective, is essentially a problem of the aesthetics of playability and little can be done to address this personal perception. Earthworm casts only become a critical problem for the player when the density reaches such a level that they adversely affect ball role, and so fundamentally impacting the quality of the sport being played. For the golf course manager, the problem runs deeper than this: as well as aesthetics, it is also an issue of the management of soil health. Completely denuding the soil of earthworms would obviously remove problems of cast formation, but this approach has considerable disadvantages for the soil's productivity and structural stability. Earthworms are key ecosystem engineers and play significant roles in both the carbon and nitrogen cycles, as well as the majority of other biogeochemical soil processes (Curry 1987; Boag *et al.* 1997; Curry 1998; Muldowney *et al.* 2003; Postma-Blaauw *et al.* 2006). The actions of a healthy and active community of earthworms in the soil therefore increases the availability of both macro and micronutrients to the growing turf, as has been demonstrated by experiments carried out in the neo-tropics (and elsewhere) where earthworms have been introduced to pasture land to increase the productivity of these soils (Lee 1985; Decaens *et al.* 2003). The relationship between the size and composition of the soil microbial community and the productivity and soil health of grasslands has also been extensively studied (Lovell *et al.* 1995; Bardgett *et al.* 1997; Bardgett *et al.* 1999; Bardgett and McAlister 1999; Hagley 2002; Grayston *et al.* 2004; Kuan *et al.* 2006; Cole *et al.* 2006) but this information on earthworms is infrequently relayed to golf course managers (*personal communications*).

The principal aims of this research were to:

A. Quantitatively investigate the ecology of earthworms on golf courses so that interpretation of both soil and environmental parameters may be used to predict both activity and diversity of earthworms; and

B. To develop earthworm casting controls on selected surfaces of golf courses through the application of the knowledge generated from this research.

The review of the literature in Chapter 1.3 identified key areas requiring research where the understandings of processes involved with the ecology of earthworms on golf courses was lacking. It was imperative that a clear understand of the interactions of earthworms with its habitat were clearly defined before attempts to implement casting control strategies are made. This clarification of earthworm behaviour on golf courses therefore directed three overlapping strands of research which could then be developed into a method for control (Figure 10.1). By studying these interactions in considerable depth it was possible to push scientific understanding beyond implied interactions. This knowledge can be disseminated into practical information available to golf course managers, beyond anecdote, and create a sound scientific basis for controls of earthworm casting.

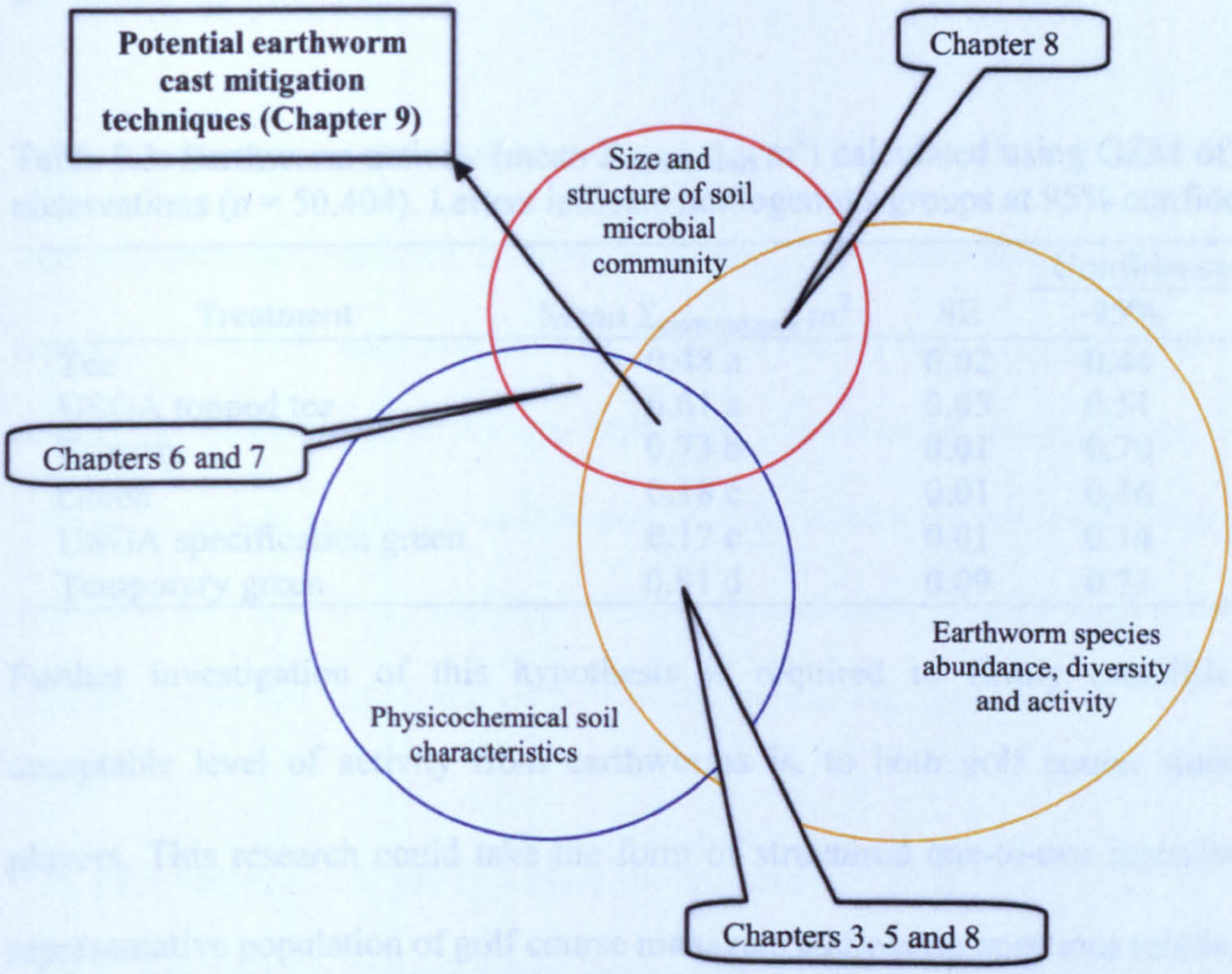


Figure 10.1: Venn diagram indicating the relationships between areas of research towards an environmentally sustainable control of earthworm casting on golf courses.

10. 2. Acceptable levels of earthworm casting activity on different areas of golf courses

The surveys carried out over two years at five golf courses in Bedfordshire and Buckinghamshire (as described in Chapter 3), with a range of play standards, showed that each surface investigated had different associated earthworm activity (Table 3.3). This level of activity has been commercially acceptable at these golf courses during the two years of the study. At the start of the survey at each golf course, the head green keeper was requested not to modify his behaviour in relation to earthworm control (or any other maintenance practice). It is therefore hypothesised that the observed annual levels of earthworm activity recorded here are the maximum

permissible levels with regards to paying customers perception of the playability of a golf course.

Table 3.3: Earthworm activity (mean $\Sigma_{casts+smears}$ m^2) calculated using GZM of all survey observations (n = 50,404). Letters indicate homogenous groups at 95% confidence.

Treatment	Mean $\Sigma_{casts+smears}$ m^2	SE	Confidence Interval -95%	Confidence Interval +95%
Tee	0.48 a	0.02	0.44	0.51
USGA topped tee	0.61 a	0.05	0.51	0.69
Fairway	0.73 b	0.01	0.70	0.75
Green	0.18 c	0.01	0.16	0.20
USGA specification green	0.17 c	0.01	0.14	0.20
Temporary green	0.91 d	0.09	0.73	1.08

Further investigation of this hypothesis is required to firmly establish what an acceptable level of activity from earthworms is, to both golf course managers and players. This research could take the form of structured one-to-one interviews with a representative population of golf course managers addressing questions relating to:

- Their perceptions of the intensity of the problem that earthworms present.
- What they consider to be the maximum permissible levels of earthworm activity on different surfaces of golf courses.
- Their attitude towards the implied relationships between the soil physicochemical parameters and earthworm casting activity.
- Their attitude towards the suggested sustainable environmental engineering solutions presented in this thesis (Chapters 8 and 9).

The perception of golf players could be addressed through rapid on-the-spot questionnaires at golf courses. These surveys could take the form of a range of photographs showing increasing densities of earthworm casts on a variety of surfaces

with the participants indicating the density above which they deemed casting to have reached an unacceptable level.

10. 3. Earthworm species abundance and diversity in relation to their control on golf courses

The experiments conducted as part of Chapter 5 provide insight to the community structure of anecic earthworms from golf courses. These findings however do not present an obvious method of either biological control or an environmental engineering solution to reduce earthworm cast formation. The most abundant earthworms recovered in the surveys are all capable of producing surface casts to some extent and therefore must be considered concurrently when proposing control methods.

The wide range of soil pH that these earthworms are adapted to living in means that control mechanisms proposed by Baker *et al.* (2000) whereby earthworm casting is eliminated through soil acidification will never be totally successful (Figure 10.2). Turf grasses will be unsuccessful at pH less than 3 or greater than 10, the extremes at which earthworms are unlikely to be present. The relationships between earthworm species diversity and the size of the soil microbial community also implies that a greater causal understanding of the relationships between the different trophic levels within the soil of golf courses might highlight potential drivers to effect changes in earthworm casting behaviour through environmental manipulations.

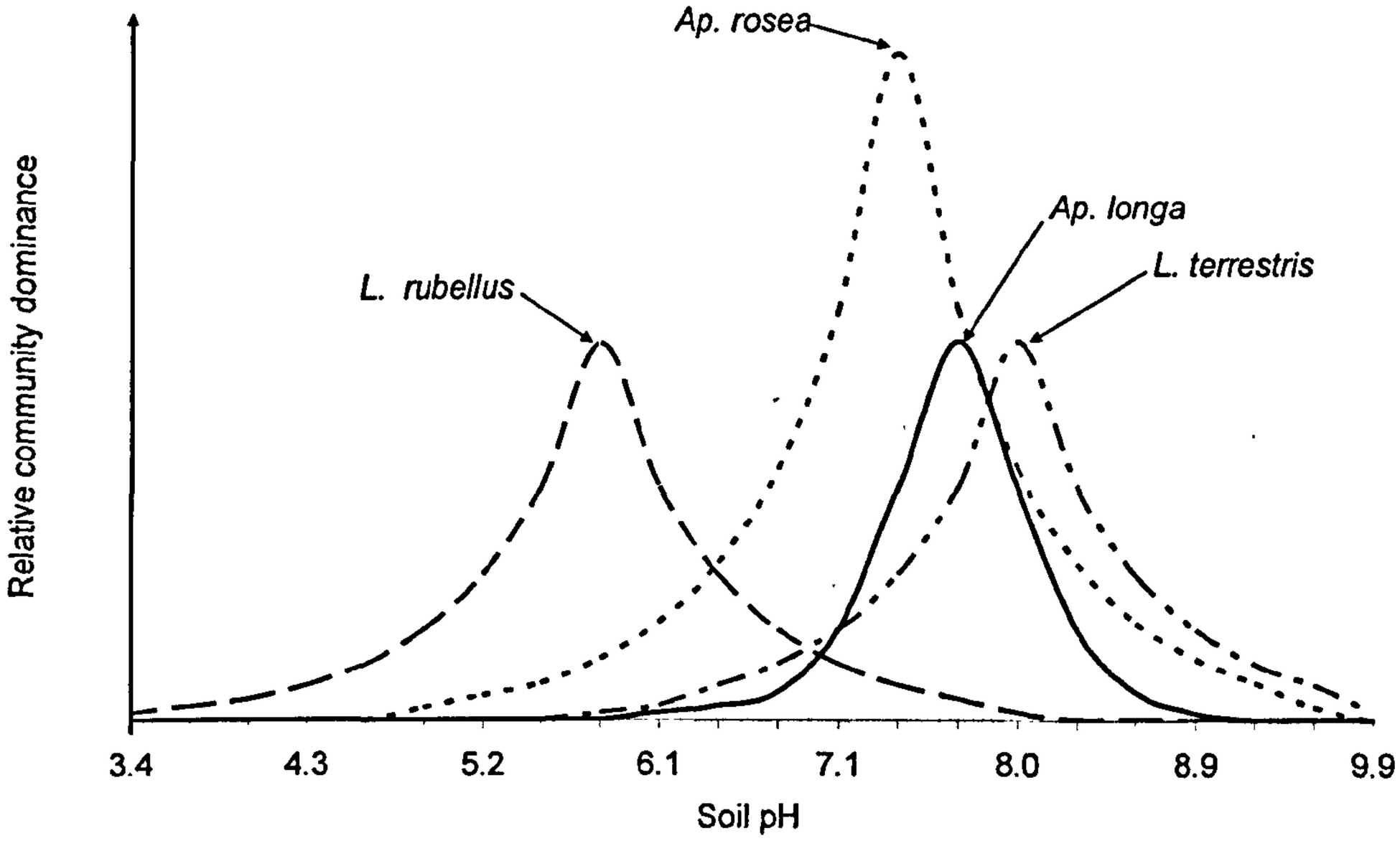


Figure 10.2: Conceptual diagram of soil pH ranges of earthworm species found in highest abundance on golf courses (scaled by relative abundance of surveyed golf courses, assuming a normal distribution)

Making artificial alterations to the community structure, e.g. performing mustard extractions on a large scale with removal and disposal of earthworms, is both ecologically unwise and potentially futile. Spatial community analysis of earthworms in the neo-tropics has shown significant spatial stability of earthworm communities (Jimemez *et al.* 2006). Removing anecic earthworms from the golf course system would mean they no longer present a problem with surface casting. However, other earthworms of the same, or different species are likely to replace those removed; filling the same role within the ecosystem and thus filling the anecic earthworm niche. Research focusing on *Ap. longa* suggests that the most dominant species in the community will out-compete all other earthworms present (Baker *et al.* 2002). Where community structures have been altered by chemical interventions the species remaining

with the greatest adaptability will dominate over others that have more specific habitat requirements. Once removed, if earthworms failed to re-colonise the soil, the effects are unclear. The removal of a keystone species (or group of species) may result in the collapse of nutrient cycling in the turf grass system. Such a problem could be avoided through ecologically sound management, supplying the soil and plant systems with the nutrients that they lack.

A significant linear relationship was inferred in Chapter 5 between species diversity (Simpson's D) and earthworm casting activity. Using the equation of this line (see page 125) and the maximum and minimum values of $\Sigma_{casts+smears}$ m^{-2} recorded over the two year quadrat survey, the range of species diversity of each surface can be estimated. Due to the disparity in scales of observation between these two experiments it is not appropriate to make estimations of error. No extrapolation has been made from this equation; the results derived were within the range of the original data.

The broad indication of these findings implies that the species diversity of both standard and USGA greens is similar, and distinct from standard and USGA topped tees, which are also similar. The fairway and the temporary green also have the same range of species diversity, which extends to a greater value than either those of the greens or the tees.

Table 10.1: Estimated species diversity (Simpson's D) on different areas of golf courses based on findings in Chapters 3 and 5

Treatment	Estimated range of Simpson's D
Standard tee	< 0.01 – 2.93
USGA tee	< 0.01 – 2.93
Fairway	< 0.01 – 4.13
Standard green	< 0.01 – 1.73
USGA green	< 0.01 – 1.73
Temporary green	< 0.01 – 4.13

10. 4. Microbial community size and structure on golf courses, related to the habitat of earthworms

The soil microbial community has a major impact on the earthworm populations that a soil can support as they represent the direct resource for metabolic sugars and proteins (Lee 1985; Bonkowski *et al.* 2000). Where there is a larger, more diverse microbial community, logically there is a greater food resource for the earthworms. Optimal foraging models, such as the marginal value theorem (Charnov 1976), dictate that the size and quality of the food supply will affect the feeding behaviour (and so by default casting) of earthworms in the golf course system. This concept is clearly enforced by the findings of Chapters 3 and 6, (Figure 6.4a and Table 3.3). Canonical correlation analysis (CCA) carried out in Chapter 6 showed that there is a similar relationship with earthworm casting activity on different types of surface and the negative to positive orientation of samples in the CCA. From these data it is impossible to resolve if it is the earthworms which are affecting the habitat or the habitat that is affecting the earthworms. It is unlikely to be a clear distinction of either the physical characteristics or the soil microbial community structure that affects these distributions as PCA of these factors separately do not project useful mechanisms on to the casting behaviour outlined in Chapter 3. In light of the evidence presented here, it is hypothesised that the

physicochemical factors which have the greatest impact through the soil flora and the abundance and quality of food that they can provide for the earthworm population would be the dominant component in the analysis.

Bardgett and McAlister (1999) reported significant differences across management gradients, but no effects in short term studies of temperate meadow grasslands. The investigations of both Chapters 6 and 7 also show this: a pooled sample taken to a depth of 225 mm did not show management related effects based on this single time point of survey. A golf course is analogous to highly intensively managed temperate grassland, and even with the large scale differences of management, no significant effects were recorded in the microbial community structure. Analysis carried out in Chapter 7 clearly demonstrated that management related effects were apparent in the upper 75 mm layer of the different playing surfaces. Different management strategies for areas of play on a golf course must therefore only exert a localised, shallow effect on the soil microbial community.

Without destructively sampling each golf course surface, it is impossible to determine the vertical distribution of earthworms in golf courses. Laboratory based experiments indicate that earthworms are predominantly at shallow depths within the soil profile (see Chapter 8). The earthworm community of golf courses is likely to be predominantly active in the top 75 mm of the soil, where there is a greater availability of food. The differences in microbial community present may also be interacting with the earthworm population distribution. Whether the microflora or macrofauna is having the causal effects is impossible to determine from these experiments. The earthworm community

is considerably more motile than the soil microbial community and as such can migrate to find the most suitable and sustained food sources. As such, it is likely that the soil microbial community commands both the size and the structure of the soils earthworm population. Clear relationships can be seen between earthworm casting activity (Chapter 3.3) and areas for play on the golf course which support significantly higher microbial populations: the fairways that reported the highest $\Sigma_{cast+smears}$ m^{-2} also has the highest microbial biomass. These two data sets cannot be linked statistically however, empirically they suggest that size and structure of the microbial community influences the cast frequency of earthworms present within the soil. Measurements of microbial community size and structure would be necessary at a range of different golf courses taken at the same time as an assessment of the earthworm population. These measurements would either have to be local to where samples for microbial analysis were taken using mustard extraction or via a quadrat survey assessing $\Sigma_{cast+smears}$ m^{-2} on each surface of the whole course. Ideally this experiment would also have a temporal dimension; however, this could be avoided by sampling when earthworm activity is at its greatest. These data could then be used to elucidate the links in the complex soil ecosystem and food web found in golf course soil, or any grassland ecosystem in general.

10. 5. The vertical distribution of earthworms in the constructed soil profiles of golf courses

Earthworms are very adaptive and capable of surviving in soil conditions that are frequently reported as being ostensibly hostile environments to them (Baker and Whitby 2003). In glasshouse based microcosm experiments (Chapter 8) where earthworms were purposely introduced into representations of certain play surfaces found on golf courses there was no significant migration to other microcosms with more benign soil

characteristics, as would have been predicted based on the research of others (Baker and Whitby 2003; Williamson 2004; Williamson and Hong 2005). These experiments indicate that the physical constructions of the anthropogenic soil profiles of play surfaces on golf courses does affect earthworm cast formation. Surfaces that include a high proportion of sand had an increased frequency of casting. As suggested in Chapter 3 and 8 it is hypothesised to be as a result of the different calorific value of earthworm available food. Earthworms in very sandy soil matrices have to ingest greater volumes of soil in order to receive an equivalent calorific intake when compared to high nutrient status soils, containing generally a greater proportion of organic matter (earthworm available food). Increased casting rates in construction matrices with a high proportion of sand may be as a result of the predominant soil inter-particle bonding forces being weak adhesive bonds which are broken with greater ease than cohesive bonds, such as those which form between clay particles. The dominance by these weaker bonds means that both burrow structure and surface casts are less physically stable, more liable break down, with soil particles falling into the earthworm's burrow, requiring fresh evacuation and surface casting in order to maintain a permanent burrow structure.

The changes to earthworm vertical distribution that were caused by the inclusion of USGA rootzone to the construction profile explains some of the effects recorded by Williamson (2004) with respect to earthworm control through top-dressing. It also suggests this method of earthworm control would never be completely effective, and has the potential to increase earthworm casting in the long term. While earthworms may not be physiologically comfortable living in high sand content soils such as USGA rootzones this soil was not so hostile that they left this open experimental system.

Photographic interpretation of microcosms (Figures 8.4c & d) showed that the gravel band frequently used to aid drainage on greens (USGA Green Section Staff 2004) is not a physical barrier layer to earthworms as perceived (*M. Jones, National Turfgrass Foundation & various golf course managers; personal communications*). These experiments would suggest that earthworms are capable of passing through this layer and it affords no protection from endemic anecic earthworm migrations from the underlying parent soil to the constructed soil profiles above. Hand sorting of these microcosms showed that earthworms were not predominantly found at a depth below the gravel layer, in both the standard green and USGA greens (depth > 260 mm) and this would seem to ratify the industry's assumptions. However, burrow structures were evident both above and below in the standard green and only below the gravel in the USGA green treatment implying that the gravel band does not represent an obstacle to *L. terrestris*.

Further quantitative investigation is required to clarify the effect of gravel drainage layers on earthworm vertical distributions. Recent technological advances of tagging individual earthworms with visible elastomers (Butt and Lowe 2006) could be used in such an experiment. This technique could be used to clarify the migratory behaviour of the earthworms in similar experiments to those described in Chapters 8 and 9. A critical difference being however, that interactions of earthworm behaviour both vertically through the soil and horizontally, between treatments could be investigated. This could be achieved by introducing earthworms both above and below the gravel band in a microcosm system followed by observation of casting behaviour. In such experiments if

there was no difference in casting frequency on all treatments it would indicate that the gravel band did not present an obstacle to the endemic earthworm population. This information would assist in understanding where earthworms migrating into new surface constructions on golf courses are likely to come from, both horizontally and vertically.

10. 6. The application of knowledge to environmentally sustainable solutions to prevent earthworm surface casting

It is important to note that care must be taken when using, and scaling up the models proposed. One of the assumptions that must be made when using either of these models in controlling earthworm casting behaviour is that over the whole system the behaviour of earthworms is constant.

10. 6. 1. Earthworm cast modelling

If the model relating earthworm activity to the physicochemical parameters of the soil (see Chapter 3) can be validated then the findings are useful to golf course architects when designing both new courses and course upgrades/modifications. Knowledge of the relationships between the anecic earthworm activity and the range of potential materials that can be used in construction could be used in decision making processes. The costs for green-keepers associated with reducing earthworm activity on specific surfaces could be reduced, through the specification of a more appropriate soil for use in constructions. This would then lead to an increase in overall course playability. Where whole new golf courses are being constructed a detailed soil survey focusing on the soil parameters highlighted by Equation 3.2 could be used in both site selection and the specific layout of the course. Selecting areas that are likely to have the lowest

earthworm activity for greens and tees would maximise the potential playability for the golf course with respect to earthworm activity.

By monitoring the daily rainfall and evapotranspiration, estimation of when earthworm activity is likely to reach a problem level is possible. This could greatly increase the efficiency of the timing of currently licensed chemical earthworm controls. A more complete understanding of the effects of rainfall (and therefore irrigation) on the activity of earthworms in relation to evapotranspiration is possible from this model. Conscientious management of tees and greens in relation to predicted weather patterns could result in a prolonged period at which earthworm activity is below the threshold of concern to both the golf players and the course managers. This could in turn reduce the workload for the green-keepers through a reduction in vermicidal spraying and the use of other maintenance practices in preparation of mowing, such as switching or brushing. The net result of a tight management regime dictated by these parameters would be an increase in environmental sustainability of the golf course.

The two most important variables effecting earthworm activity identified (% total organic carbon and C:N) can be manipulated through physical intervention of maintenance practices, thus potentially reducing levels of earthworm activity. The % total organic carbon content of the soil, intimately related to thatch build up, is routinely reduced through hollow tinning. The C:N ratio of the soil can be changed through the application of nitrogen based fertilizers. Through interpretation of both of these variables, based on laboratory testing, on each surface it should be possible to identify when earthworm activity is likely to become a problem. There are also clear implications to earthworm activity to the use of carbon rich 'soil conditioners'. These

'soil conditioners' result in an increase in soil microbial biomass and therefore increase the % total organic carbon in the soil. At almost all C:N ratios Figure 3.5 suggests that this will increase the activity of earthworms. Understanding the relationships between earthworms and this wide range of soil physicochemical and environmental variables means an increase in fact based options for the green-keepers of the five golf courses studied here to control earthworm cast activity. These principles could be applied to other golf courses if data was collected to validate the proposed model.

10. 6. 2. Physical exclusion

The experiments pertaining to the physical exclusion of earthworms requires considerably more research before it can be scaled up to a whole golf course study. Research to elucidate a causal mechanism behind the control effects recorded is required so that the field scale research can be fully parameterised. Only once the extensive set of experiments described in Section 9.3.3 has been conducted would it be suitable to conduct golf course scale trials. Trials at this scale might make use of golf courses such as Woburn Golf and Country Club, where there are three golf courses of similar standards in close proximity. Nine of the eighteen tees on each of these golf courses could then be re-constructed, including the most appropriate barrier layer. The remaining nine tees could be left as controls. A survey of earthworm cast frequency could then be carried out over a number of months or years to ascertain the effect of the barriers inclusion. If this method proved to be effective on the tee area of golf courses, due to the depth of the membrane, at 200 mm, it would mean that all maintenance techniques could continue un-altered. Despite the potentially high financial costs of initial installation, once installed it would require no further maintenance costs relating to earthworm control.

10. 6. 3. Soil biological control

Research described in Chapter 9 has shown that a reduction in earthworm cast frequency can be achieved by manipulating the soil microbial community on USGA greens. Considerably more research is required for this solution, to establish the long term effects of the application of glucose substrate to USGA rootzone materials. This solution represents significant potential as a control mechanism for greens of this construction. It is unlikely to be transferable to other areas of golf courses were the calorific value and textural characteristics of the soil are closer to the optimal requirements of the earthworm. A significant disadvantage of this control method is that applications of glucose substrate must be made on a regular basis in order to maintain the size of the soil microbial population. A further suite of experiments where by the percentage of organic matter in the USGA specification rootzone is increased may reduce the amount of 'extra' biomass required through the addition of glucose, and hence decrease the dosage rate of available carbon. Further investigation with a range of different substrates of chemical complexity may result in a better range of control or a more commercially viable solution. When implementing such a control method considerable attention would be required to the size of the soil microbial community, ensuring that the population was sufficiently large so resulting in a reduction of cast formation, rather than increasing the propensity for cast formation as discussed earlier.

10. 7. Directions for future research

Due to practical limitations it was not possible to make direct observations of species diversity of earthworms from destructive sampling on any of the surveyed surfaces, consequently only implied measures of species diversity are possible. This is further limited by the biases inherent in the mustard extraction technique with regards a potential over estimation of earthworms that have burrows open to the surface (Chapter

4). Earthworm species producing large amounts of surface casts could potentially be directly identified through DNA analysis of the cast soil. Harper *et al.* (2005) have carried out some experiments to rapidly screen invertebrate gut contents in order to measure energy transfers in soil food webs. Further research with regards to both the extraction of specific earthworm DNA fragments from the soil and the earthworm gene library would be required to make this a practical tool in the investigation of earthworm species diversity from cast material.

The following topics of research are suggested following on from this thesis:

i. Validation of earthworm casting model (Chapter 3) using a wide range of golf courses in diverse geographical locations, recording real-time local environmental data.

ii. Direct assessments of earthworm species diversity from golf courses, either through small scale replicated experimental plots where destructive sampling is possible; destructive sampling on golf courses; or advances to DNA based earthworm identification techniques.

iii. Investigation of interactions at the whole earthworm community scale between community size and structure and associated soil microbial size, activity and community structure. Understanding of these mechanisms is of potential use both for sports surfaces and agricultural systems.

iv. Development and up-scaling of earthworm casting control measures further investigating soil engineering solutions and mitigation using soil microbiological control.

v. The applications of the findings of this thesis to other sports surfaces where earthworm casts are problematic, e.g. crown green bowls or cricket.

10. 8. Concluding remarks

This study has advanced the understanding of the relationships between earthworm ecology and golf courses, deriving mechanistic understandings between the relevant components of the system. The following statements with regard to the ecology and control of earthworms on golf courses can be made:

- There is significant interaction between earthworm casting activity and the physicochemical characteristics of distinct play surfaces found on golf courses. The most significant variables determining the distribution of activity were: % TOC, C:N, % Sand, CEC and % Silt, which were mathematically related to earthworm activity measured as $\Sigma_{casts+smears}\ m^{-2}$ (Chapter 3).
- Earthworm casting activity varies bimodally with season; greater levels of activity were recorded during the spring and autumn. This variation is mathematically related to mean rainfall and mean evapotranspiration (Chapter 3).
- The most dominant species of earthworm found on fairways, with burrows that are continuous to the surface and capable of forming surface casts, are *Aporrectodea rosea*, *Lumbricus rubellus*, *Aporrectodea longa* and *Lumbricus terrestris* (Chapter 5).
- The species diversity of anecic earthworms on the fairways of golf courses can be related to the size of the microbial community supported by the soil. The species diversity within the inherent microbial community can also be related to surface activity of earthworms (Chapter 5).

- The phenotypic microbial community structure of golf course surfaces (relating to earthworm feeding preferences and activity) are distinct for each play surface of a golf course, irrespective of its geographical location. This indicates there may be a relationship between phenotypic microbial community structure and earthworm casting activity (Chapter 6).
- The community size and structure of the soil microbial community found on golf courses surfaces are significantly different in the topmost 75 mm of the soil depending on the management applied to maintain the playability of the surface. This suggests that the vertical distribution of earthworm will be related to the vertical changes in resource availability, e.g. the microbial community (Chapter 7).
- There are no strong effects of construction techniques of the soil profile on earthworm vertical distribution or casting behaviour. When earthworms are forced to inhabit soils which have been reported as hostile, i.e. rootzones containing < 80% sand, they are capable of survival and the formation of casts (Chapter 8).
- The inclusion of a physical barrier to earthworm movement within the soil profile has an effect on the rate of cast formation. However from the experiments carried out no mechanism could be determined for this effect (Chapter 9).
- Manipulation of the size of the soil microbial community affects the rate of earthworm cast formation, where the soil has an increased microbial biomass there was a lower rate of casting (Chapter 9).

Within the time frame of this research no conclusive mechanism of controlling earthworm cast formation on golf courses has been derived, but several clear indications for the direction of future research have been highlighted with significant potential to result in an effective control or mitigation mechanisms. Further research has strong potential to produce an environmentally benign and sustainable means of reducing earthworm casts.

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Appendix I: Dissemination

Peer reviewed publications

Bartlett, M.D., Harris, J.A., James, I.T., Ritz, K. 2006. Inefficiency of mustard extraction technique for assessing size and structure of earthworm communities in UK pastures. Soil Biology and Biochemistry 38: 2990 – 2992.

Bartlett, M.D., Harris, J.A., James, I.T., Ritz, K. Interactions between microbial community structure and the soil environment at five UK golf courses. Submitted to Soil Biology and Biochemistry.

In review

Bartlett, M.D., Harris, J.A., James, I.T., Ritz, K. Speciation of anecic earthworms on five English golf courses. - Applied Soil Ecology.

Bartlett, M.D., Harris, J.A., James, I.T., Ritz, K. Estimating species richness of earthworms on golf courses and implications for innovating environmentally benign control methods. - Acta Horticulturea

Trade press publications

Bartlett, M.D. 2005. Earthworms, friend or foe. Pitchcare magazine, Oct-Nov 2005.

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Short communication

Inefficiency of mustard extraction technique for assessing size and structure of earthworm communities in UK pasture

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Abstract

An assessment of the efficiency of the mustard extraction method to quantify the total earthworm community structure on UK earthworms was carried out on a permanent pasture in Bedfordshire, UK. Earthworms were collected using mustard extraction and control treatments. Numbers and community structure of worms expelled from soils after surface applications of expellants were determined, and underlying soil from each replicate was hand-sorted to recover residual earthworms. The mustard-based treatment was the only method to expel earthworms to the surface, and 35.7% of the extant population emerged. The apparent earthworm community structures expelled indicated that mustard extraction was biased towards large, sexually mature anecic earthworms. This is likely due to the connectivity of burrows of these earthworms to the surface and hence a biased incursion of mustard solution down such channels. The mustard extraction technique is therefore inappropriate where accurate assessment of earthworm communities is required.

Keywords: Anecic Earthworms; Mustard extraction; Non-destructive sampling

There are inherent problems with sampling in order to make accurate assessments of earthworm populations, since the techniques used must be invasive to the earthworms' natural environments. Since the first published scientific investigation into earthworms (Darwin, 1883), two approaches to sampling these organisms have predominantly been used, based on chemotactic and physically destructive methods. Earthworms move in predictable ways in response to chemical gradients and this behavioural trait can be used to force earthworms to the surface by drenching the soil with an irritant chemical. Hand-sorting soils requires the physical destruction of the soil volume, and whilst definitive, has a drawback in the length of time required to produce a reliable result. Raw (1959) pioneered a means of chemotactic extraction using formalin. Formalin is a highly toxic and carcinogenic compound and thus not admissible for such environmental application.

Another less-toxic formulation shown to have an expellant effect on earthworms is mustard flour solution. Allyl isothiocyanate is an alkaloid that is produced through the enzymatic breakdown of glucosinolates in mustard flour and is an irritant to the mucosal membranes of earthworms (Zaborski, 2003). The use of mustard solutions was piloted by Gunn (1992). Lawrence and Bowers (2002) assessed that mustard extraction can account for 98% of the total worm biomass of a soil sample and 83% of species when compared to handsorting. The method has been noted for being more effective at expelling species that have their burrows open at the surface (e.g. *Lumbricidae* spp.; Springett, 1981), and thus the technique may be biased to such species.

Mustard extraction is widely used, and an international standard is defined (ISO, 2000). However, no formal assessment of its effectiveness with UK earthworm species has been carried out. In this study, the extraction efficiency of mustard solution was evaluated under the hypothesis that a representative proportion of the extracted earthworm community will be expelled when mustard solution is applied to a defined surface area of turf.

Experiments were conducted in a 200 m^2 area of pasture land at Silsoe College Farm, Bedfordshire. The soil is a

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sandy loam (pipette method) of pH 6.7 (in H_2O) and CEC 24.4 cmol kg^{-1}. Sampling was conducted on the 31 March and 1 April 2005. Three treatments were used: no surface application (denoted Control); water only (denoted Water); mustard (Colman's, Norwich, UK) solution at 6 g l^{-1} (denoted Mustard). These treatments were selected, as earthworms are terrestrial species and thus may have a chemotactic response to either mustard solution or water. Each treatment was applied using the ISO 11268:1999 protocol (ISO, 2000). Soil conditions at this time were such that all solutions percolated completely within 12 min. Seven replicates of each treatment were applied to the soil surface bounded within a 0.25 m^2 mild steel ring pressed 10 mm into the soil surface, in a completely randomised design. All expelled earthworms were collected over a 30 min period following application. Subsequent to this, the soil below the ring was excavated to 0.2 m. The soil from each replicate was hand-sorted in the field to recover all residual earthworms. Earthworms recovered from each treatment were categorised as expelled or residual. The total population was defined as the sum from both hand-sorting (residual) and surface extraction (expelled). The Control was taken to represent an unbiased sample of the extant earthworm community. Earthworms were stored in oxygenated water at 2 ± 0.5 °C prior to speciation. Adult earthworms were identified by the presence of a clitellum. Earthworms without a clitellum were identified where possible and recorded as juveniles. Community structures were compared using principal component analysis and one-way analysis of variance of the resultant components.

A total of 1117 earthworms were recovered from all treatments in the experiments, of which 39.7% were adults. There was no significant difference in the total number of earthworms recovered in any of the treatments ($p = 0.14$). The Mustard treatment was the only method to expel earthworms to the surface with 35.7% of the total population emerging (Table 1). Seven different species were represented with both adults and juveniles apparent from all but *Lumbricus festivus*, where only adult earthworms were found. Four per cent of juveniles were not identifiable (Table 2). No aestivating earthworms were recovered from any of the treatments.

Table 1
Mean number of expelled, residual and total earthworms from each treatment (n = 7)

Treatment	Expelled (E)	S.E.	Residual (R)	S.E.	Total (T)	S.E.
Control	00.0 a		67.9 b	7.61	67.9 a	7.61
Water	00.0 a		43.0 a	8.57	43.0 a	8.57
Mustard	17.4 b	2.54	31.3 a	8.57	48.7 a	9.32
F	47.0		4.87		2.22	
p	<0.01 *		0.02 *		0.14	

Values not followed by same letter significantly different within columns using Tukey test.

Table 2
Frequency of earthworm species and total number of earthworms recovered, ranked according to associated loading values for first principal component

Earthworm species	Sexual maturity	Expelled (*n* = 7)		Total (*n* = 21)		PC1 loading
		Mean	S.E.	Mean	S.E.	
Aporrectodea rosea	Adult	3.57	0.57	9.57	1.07	0.067
Lumbricus terrestris	Adult	2.29	0.18	4.43	0.65	0.065
Lumbricus rubellus	Adult	1.71	0.75	2.00	0.58	0.053
Allolophora chlorotica	Adult	0.86	0.46	2.71	0.84	0.013
Lumbricus rubellus	Juvenile	0.29	0.29	3.86	0.80	0.002
Lumbricus festivus	Adult	0.00	0.00	0.57	0.43	0.000
Lumbricus castaneus	Juvenile	0.57	0.43	4.14	1.45	−0.002
Aporrectodea caliginosa	Adult	0.86	0.70	3.29	0.81	−0.006
Unidentifiable	Adult	0.43	0.43	1.71	0.68	−0.006
Lumbricus terrestris	Juvenile	0.43	0.43	2.43	1.04	−0.014
Lumbricus castaneus	Adult	1.00	0.44	3.71	0.92	−0.016
Allolophora chlorotica	Juvenile	1.14	0.86	3.71	1.04	−0.026
Aporrectodea caliginosa	Juvenile	0.29	0.29	9.00	1.90	−0.046
Aporrectodea rosea	Juvenile	4.00	1.00	16.71	1.63	−0.084

In the community structure analysis, the first three principal components (PC) accounted for 71% of the variation between samples. Only PC1 showed significant treatment effects ($p = 0.02$). The loading values of PC1 show that juvenile *Aporrectodea rosea* and *Ap. caliginosa* were heavily negatively loaded while adult *Lumbricus rubellus*, *Ap. rosea* and *L. terrestris* were positively loaded (Table 2).

The number and species of earthworms found during this survey were consistent with that of other studies in the UK (Edwards, 1996). The proportion of adults to juveniles (40:60) was also consistent with other findings for spring-time (Fraser et al., 1996).

These experiments demonstrate some problems with the assessment of the whole earthworm community using mustard extraction. Direct comparison of community size gave misleading assessment of earthworms present when using a chemical extraction of this type. These differences indicate that not all earthworms respond in the same way to mustard extraction, broadly supporting the work of Chan and Munro (2001) who report that the anecic earthworms in Australia (*Anisocheatea* sp.) respond to mustard extraction, however *Ap. trapezoids*, an endogeic species, were completely unaffected.

The significant difference in apparent community structure between expelled and total fractions (Table 2) means the hypothesis that a representative proportion of the extant earthworm population will be expelled by mustard extraction in comparison to hand-sorting must be rejected on the following bases:

1. *Body size*: There is a trend in size of organism, larger loading values from PC correspond to earthworms with a larger surface area, and smaller loading values are represented by earthworms with a lower surface area.
2. *Earthworm maturity*: A trend in maturity as loading values of PC become more negative, juveniles being negatively loaded and adults positively. These data confirm that adult and juvenile earthworms of the same species respond differently to mustard solution.
3. *Surface active earthworms*: *Ap. rosea*, *L. terrestris*, are both anecic earthworms, with permanent burrows open to the surface. *L. rubellus* is endogeic, with shallow burrows open to the surface. These three species are represented as the largest loading values on PC1.

These results can be explained in terms of the pore structure of the soil. The soil matrix is a complex non-uniform structure, with a range of pore sizes found between the solid constituents. Burrowing and subsurface casting of earthworms has been shown to increase the size and distribution of pore spaces (Gorres et al., 2001). The burrows of all earthworms will form macropores proportional to the body size of the earthworm that created it. The habit of anecic earthworms also means that macropores associated with them will be open at the surface. Smettem (1992) showed that the presence of anecic earthworms, producing such macropores, has a significant effect on the hydraulic conductivity of a soil. The preferential flow of water through the soil means that earthworms in the soil will not all have the same exposure to the mustard solution, especially over the short term. Earthworms in macropores that are large or well connected to the surface will be exposed to the mustard solution first, and hence are more likely to be expelled.

Both endogeic and the majority of epigeic earthworms will also be exposed to the mustard solution, but over a longer period of time as it percolates thorough the soil via smaller pore networks. Endogeic earthworms may not respond in the same way as other earthworms to the mustard solution. The life-history of endogeic earthworms is such that they tend to prevail below the surface of the soil. Therefore, even if the mucosal membranes of these earthworms are exposed to the allyl isothiocyanate from the mustard solution, their behavioural response is not necessarily to migrate to the surface, and migration of these earthworms horizontally through the soil may be a more likely response.

These data have implications for the interpretation of data from mustard extraction surveys. Sampling using this method will be biased towards anecic earthworms and not the earthworm community as a whole, and due account should be taken of this in planning and reporting surveys.

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EARTHWORMS Friend or foe!

Horticulturalists the world over have been encouraging earthworms in the soil for years, but the turfgrass industry has always seen them as a pest. Why is it that groundsmen and greenkeepers hate and fear earthworms so much? Should they really be that concerned about all of them? How can they control them?

Cranfield Centre for Sports Surfaces has taken on the challenge to answer these questions.

EARTHWORMS eat soil, and lots of it. They can eat up to 30 times their own body weight every day which passes out of the other end as fertile, well structured aggregates. There are normally about 300 earthworms below a square metre of turf, and the burrows that earthworms create and live in improve the drainage of soil. Earthworms also eat thatch and other organic material in the soil so they can reduce thatch build up. Earthworms help to complete nutrient cycles in the soil, reducing the need for inorganic fertilisers. A healthy earthworm population has the same effect as adding 100 kg of nitrogen fertiliser per hectare every year because they free nutrients from the soil.

Not all earthworms behave the same way, only deep burrowers earthworms are a pest to turfgrass because of the casts that they leave on the playing surface.

There are 25 different species of earthworm found in the United Kingdom, but only three are deep burrowers causing this problem, the most common is *Lumbricus terrestris*. Non-surface casting earthworms are beneficial for turf growth, being responsible for the majority of the nutrient recycling and thatch breakdown.

Earthworm Habitats

Earthworms can survive in a wide range of conditions, but most earthworm activity is dependent on the quality of food available and the season. Deep burrowing earthworms will thrive where clippings are not boxed since food is always available. During the year when soil temperatures are at their lowest and highest earthworms go into a form of hibernation where they do not eat and can not move. This is why there are more problems with earthworms in the spring and autumn. The soil pH affects where earthworms are found and in strongly acid or alkaline soils earthworms are rarely seen (pH less than 4.5 or greater than 8). The soil texture will also affect the number of earthworms found; they prefer clay soils and are less frequently found in sandy soils.

Earthworms in Turfgrass

Farmers encourage deep burrowing earthworms to thrive in their fields because of all the nutrient benefits they give, but casts on the surface of turf cause groundsmen and greenkeepers several problems. Deep burrowing earthworms generate casts that spoil the appearance of the turf surface that customers have paid to play on. More than this, if casts are then pushed across the surface by mowers or trodden underfoot then they can create patches of turf that are hard to revive and can also cause problems with drainage. On sandy soils this can also blunt mower blades. Another problem is that deep burrowing earthworms can move weed seeds from within the soil to the surface where they are able to germinate. Earthworm casts provide ideal conditions for germination; they are loosely packed, moist and full of nutrients. Even if the seeds are not in the soil, the earthworm casts provide a perfect tilth for wind blown weed seeds to germinate.

Control of Earthworms

Historically, earthworms have been controlled chemically, killing all earthworms in the turf. The most widely used chemical was chlordane, an organochloride, now banned due to it's wide ranging toxic effects and persistence in the environment. Other chemicals such as benomyl, carbendazium, thiabendazole and thiophanate-methyl (all of which are primarily fungicides) have an effect on earthworm populations. Research has shown that thiophanate-methyl is the most effective at reducing casting. All these fungicides are considerably less effective at earthworm control than chlordane.

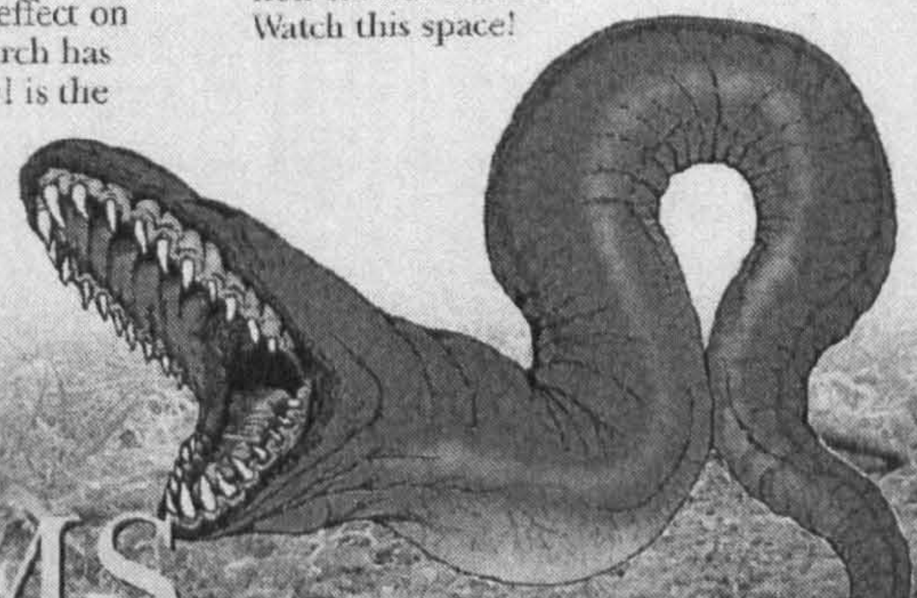
Some groundsmen and greenkeepers use a different approach, trying to make the soil unsuitable for earthworms. This is achieved in two ways: the available food is reduced, by boxing grass clippings; and the soil is acidified using sulphur-based fertilizer thus lowering pH. The disadvantage of this approach is that soil unsuitable for earthworms is also unsuitable for turf, which can struggle to grow. Prior to the widespread use of chlordane, one way earthworms were controlled was using chemicals that forced the deep burrowing earthworms to the surface. The chemical irritates the earthworms which come up to the surface. The draw back with this method is that the earthworms then need to be collected and disposed of.

The Solution?

Groundsmen and greenkeepers are always trying to strike a balance between the positive and negative effects of earthworms. Everybody wants the increased aeration, drainage and nutrient cycling advantages of having the earthworms present, but nobody wants the problems associated with casting.

Soil scientists at the Cranfield Centre for Sports Surfaces and the National Soil Resources Institute have been researching this problem, by trying to link all of the pieces of the puzzle. Focusing on golf courses they are measuring the size of the casting problem, working out what species are causing the damage and why they are there. Once these pieces are fitted together it should be clearer how the deep burrowers can be controlled and still maintain the advantages from the non-surface casters.

Watch this space!



Earthworms can survive in a wide range of conditions but activity depends on the quality of food available

About the Author:

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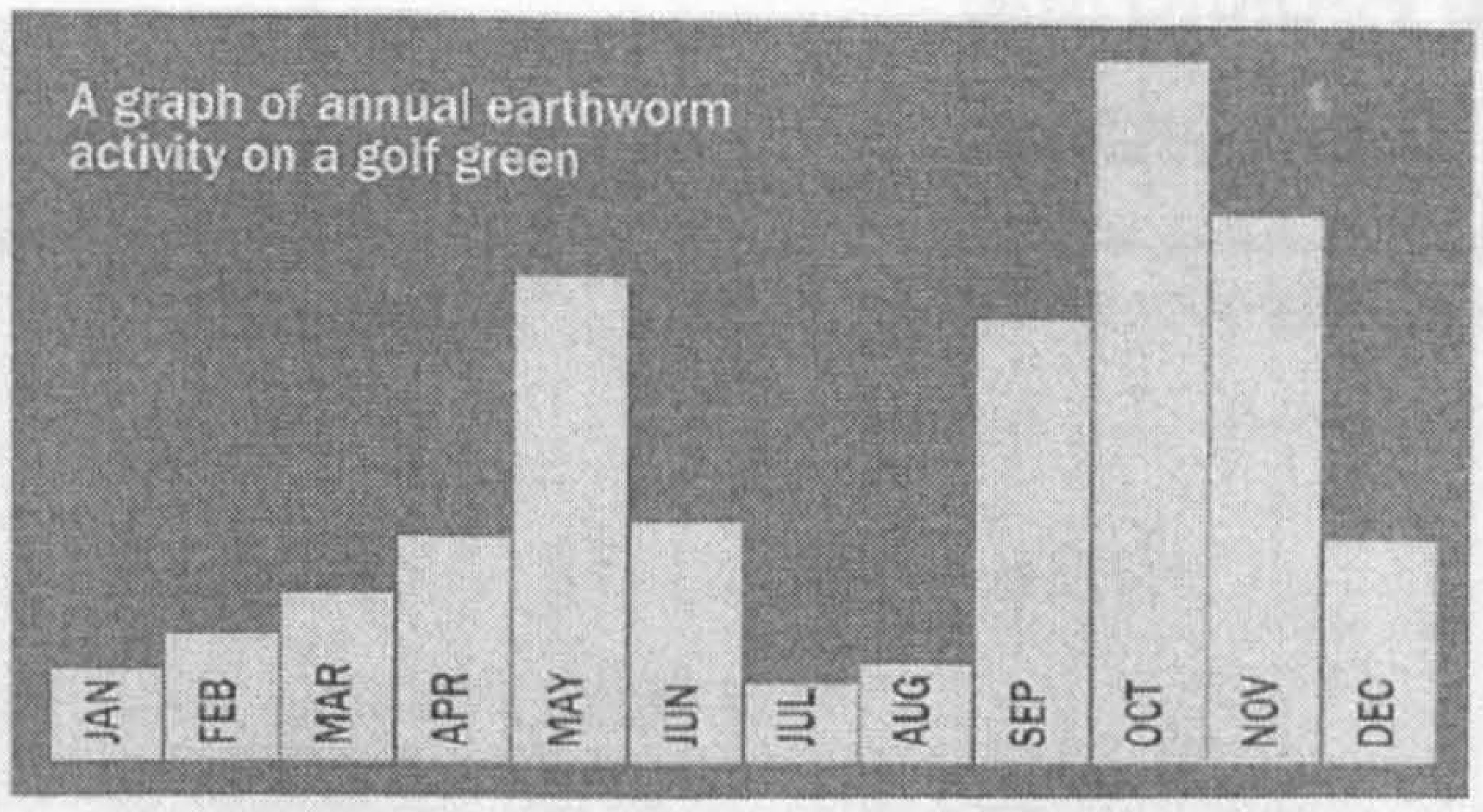
Cranfield Centre for Sports Surfaces is a centre for research and training expertise based at Cranfield University at Silsoe. It provides the MSc Sports Surface Technology degree, unique in Europe for the advanced education of industry professionals. It is also a centre for excellence in both doctoral and commercial research, working with industry bodies and the UK government to advance knowledge, and to develop safe, sustainable sports surfaces in all environments. More details are at our website www.silsoe.cranfield.ac.uk/ccss.

The National Soil Resources Institute was established in 2001 to create a unified institute with the necessary scientific expertise and research capability to focus on the long-term development of the sustainable management of soil and land resources, both in the UK and around the world. Further information about NSRI is available at: www.silsoe.cranfield.ac.uk/nsri



Know your worms!

Earthworms are very adaptable creatures and can live in a range of soil environments. Biologists divide them into three groups

Epigeic earthworms: Most commonly found in woodland environments. Not important to sports turf.

Endogeic earthworms: These earthworms form burrows that are not open to the surface and do not cast at the surface. They normally live in the top 15 to 20cm of the soil.

Anecic earthworms: These earthworms are capable of burrowing deeply and normally have burrows that are open to the surface. They feed on leaf litter that they find at the surface and mix it within the soil horizons. This type of earthworm is responsible for the formation of surface casts that cause major problems for sports turf.

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Interactions between microbial community structure and the soil environment found on golf courses

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Abstract

Approximately 0.6% of the total UK land surface is occupied by golf courses, but little investigation into the biological properties of the soil under this type of amenity turf has been reported. The soil microbiota has a significant role within the majority of nutrient cycles. In order to analyse how golf course management affects the soil microbial community, an investigation of the phenotypic microbial community structure using phospholipid fatty acid (PLFA) analysis was carried out. Principal component analysis of PLFA biomarkers indicated that there were consistent relationships between the tees, fairways and greens and the soil microbial community structure. No conclusive mechanism could be demonstrated in one-way analysis with corresponding physical parameters ($P>0.05$ in all cases). Cannonical correlation analysis (CCA) using 28 PLFA biomarkers concurrently with 9 physicochemical parameters showed a highly significant relationship on different playing surfaces at all of the golf courses surveyed ($P<0.01$). The construction and maintenance of specific areas of a golf course, irrespective of geographical location, closely reflect the physicochemical status of the soil microbial habitat.

Keywords: Golf course; Microbial community analysis; PLFA; CCA; Physicochemical–biological interactions

1. Introduction

It is estimated that approximately 1500 km^2 of the UK surface mass is occupied by golf courses, representing a total national coverage of 0.6% (based on assumptions made by Terman (1997)), and a larger surface area than most other recreational facilities. In urbanised areas the proportion of amenity turf (including golf courses) has been recorded to be as high as 4% (Office of the Deputy Prime Minister, 2005). There is little comprehensive data available on the microbial community structure found in the soil of any sports surface, but particularly in relation to golf courses. Work that has been carried out has principally been conducted by agrochemical companies to support products that 'promote' the activity of bacteria and fungi, and as such are marketed as soil conditioners to improve turf grass growth (Hagley, 2002; Mueller and Kusow, 2005). Assessments on golf course of soil microbial communities have been made by the United States Golf Association (USGA), however these have predominantly been measured *in vitro* using culture-enrichment colony counting techniques (Karp and Nelson, 2004). This method has been shown to be unrepresentative of extant community structures in soils, typically isolating less than 1% of the community (Brock, 1987; Saleh-Lakha et al., 2005). The soil microbial community is a major contributor to nutrient cycling and food webs within the soil, and has been used extensively to indicate ecosystem status (Harris, 2003). Furthermore, microbes interact extensively and critically with soil fauna to drive elemental cycling and other ecosystem services. In the context of golf turf, earthworms are a significant component of the soil fauna since although they are involved in organic matter dynamics and restructuring of the soil, they can also be problematic to green keepers to whom anecic earthworm casts present problems relating to the aesthetic qualities of the turf. Casts can also cause damage to mowing equipment, blunting mower blades. There is evidence linking the quantity of soil microbial

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biomass with the presence and activity of earthworms (Doube and Brown, 1998; Gorres et al., 2001; Lavelle, 2001).

The genotypic structure of the soil biota varies over different soil types and geographical locations (Tiedje et al., 1999), however it is the interaction of each individual microbe's genotype with the environment that governs the phenotypic expression of the microbial community. It is the microbial phenotypic community structure, and its products, which have direct effects on nutrient cycling and environmental processes in the soil. By assessing the microbial phenotype expressed in the soil, and therefore the microbial community structure, relationships can be inferred about the nutrient cycles and processes that are active within the soil. Land management techniques have often been shown to alter the microbial community structure. Work carried out in arable systems has shown that crops that receive high dosages of fungicidal chemicals have an altered microbial community structure (Zelles, 1999). The majority of golf course turf is sprayed with fungicides and is fertilised, however at differing rates and frequency at different courses, contingent upon the green keeper's opinion and legislative/labelling restrictions of the chemicals. These different management practices are likely to be reflected in the microbial population found within the soil. Three primary categories of turf are present at all golf courses: tees—where each hole begins; fairways—a considerably larger area, over which the game is played; and the green—where the target hole is located. The tee and green areas of golf courses can be described as anthropogenic soil profiles. Materials are often imported from other regions (even globally) in order to construct the optimal play surfaces. The construction materials used in a USGA specification green are considerably different from a standard green, with USGA specification rootzone containing at least 80% fine sands (USGA Green Section Staff, 2004) and the standard green only making use of locally available soils for the rootzone. Consequently, such anthropogenic soil matrices below each type play surface have considerably different physicochemical properties in comparison to the surrounding parent soil. At some golf courses temporary greens are occasionally constructed by mowing areas of the fairway closely (ca. 5 mm), over seeding with fine leaved grass species such as *Festuca* spp. and subsequently maintained as a standard green. No modifications are made to the soil profile (Golf course managers; personal communication). It is hypothesised that the level of maintenance that different areas of the golf course are subjected to affects the expression of the phenotypic community structure of soil microbes. This is due to different physicochemical constraints which are implicit in the anthropogenic soil profiles constructed and maintained on each different area of the golf course. To investigate this, the following hypotheses were tested:

1. Different construction materials and maintenance regimes of different areas of golf courses will result in a different phenotypic expression from the soil microbial community due to its response to treatment (play surface) specific environmental parameters.
2. Geographically dispersed golf courses at a regional scale will have different soil microbial community structures due to variation in soil chemistry and other environmental parameters.

2. Materials and methods

2.1. Experimental design

Samples were taken from five golf courses in Bedfordshire and Buckinghamshire, UK, in early March 2005; these courses were designated A–E by their geographical distribution, from east to west. The diversity of the soil textures and other physicochemical parameters at these golf courses were wide ranging, with a range of sand content from 40% to 85% between survey sites (Bartlett, 2006). At each of the five courses, six playing surface types were identified by virtue of their different construction and maintenance technique. The difference in surface preparation and maintenance result in different 'products' to the golfer, with regards to the surfaces on which they play, these differences were used as treatments in this investigation. The play surface types are each similar in construction and maintenance procedures, irrespective of their geographical location and were as follows: Standard tee (designated T); USGA topped tee (designated UT); Fairway (designated F); Standard green (designated G); Temporary green (designated TG); USGA green (designated UG). Only one golf course (Course E) had two different types of the same playing surfaces where temporary greens were in use during the installation of new USGA specification greens.

At each golf course five randomly selected tees, fairways and greens were sampled. Soil cores were taken on each play surface from the nodes of a *W*-of-best-fit on tees and greens ($n = 5$) and were pooled in the field within each replicate. Each soil core was taken with an auger (10 mm diameter) to a depth of 250 mm. To limit root material contamination of the samples, the top 25 mm of each soil core was discarded in the field. On the fairways, a stratified random design was used, to account for the disparity in area. Ten evenly sized strata were imposed on the length of each fairway and one soil core was taken from a randomly selected position within each stratum ($n = 10$). These samples were also pooled in the field. Soil samples were stored at 4 °C for 7 days prior to sieving at field moisture content to pass through a 4 mm sieve. Samples were then frozen at −86 °C and freeze-dried using an Alpha 1–2 LD freeze dryer (Christ Freeze Driers, Osterode-an-Harz, Germany).

2.2. Laboratory analyses

An assessment of the phenotypic structure of the microbial community was made using phospholipid fatty acid (PLFA) analysis. The extraction procedure is based on

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a modified method described by Frostegard et al. (1991), Bligh and Dyer (1959) and White et al. (1979). Detection of extracted lipids was made as fatty acid methyl esters (FAMEs) using gas chromatography (Dowling et al., 1986). All samples were analysed using an Agilent Technologies 6890N gas chromatograph (GC). Injection was controlled by, and integration carried out using Agilent G2070 ChemStation for GC systems (Agilent Technologies, California, USA). The GC was equipped with a slit/splitless auto-injector and an HP-5 capillary column; 30 m length, 0.32 mm internal diameter, 0.25 μm film (Agilent Technologies, California, USA).

Standard mixtures of 32 known FAMEs (Sigma-Aldrich, Dorset, UK) were used to identify the retention time of the PLFAs of interest from each sample. Peak area from samples was recorded by integration at these pre-identified retention times on the chromatogram for each sample. The relative concentration of each PLFA was expressed on a mol% basis. This results in a proportional determination of the phenotypic microbial community structure, as such no assessment of community size can be made using this method. The following PLFA biomarkers (derived from FAMEs) were identified: 14:0; 14:1 isomer a; 14:1 isomer b; i15:0; ai15:0; 15:1; 15:0; 16:1 isomer; i16:0; 16:1ω7 c; 16:1ω7 t; 16:0; Me17:0 isomer; i17:0; ai17:0; 17:0 c; 17:1 isomer; 17:0; 17:0 isomer; 18:0 isomer; 18:2ω6 c; 18:1ω9 c; 18:1ω9 t; 18:1ω7 t; 18:1 isomer; 18:0; 19:2; 19:0 c; 19:0; 20:0.

The soil physicochemical environment of each playing surface sampled was also determined. The parameters measured were: particle size distribution, measured using the method described by Bouyoucos (1951) (sand, clay and silt (%) were measured, but silt omitted from the analysis in order to balance the canonical correlation analysis (CCA) matrix—see below); organic matter, by combustion at 450 °C (%); cation exchange capacity (cmol kg^{-1}), by pH adjusted total base saturation (ISO, 1994); pH, as a 2.5:1 water:soil slurry (MAFF, 1969); total organic carbon (%TOC), total nitrogen (%N); total carbon (%TC) and hence C:N ratio, by quantitative reduction in an elemental analyser (Vario EL, Elementar Americas Inc., USA).

2.3. Data analysis

Principal component analysis (PCA) using covariance and post-hoc one-way analysis of variance (ANOVA) was carried out on mol% data to determine ecologically relevant structures within the data. CCA was also used to determine correlations between the physical environment and the phenotypic community structure. All analysis was carried out using Statistica 7.1 (Statsoft Inc., 2005).

3. Results

3.1. Phenotypic community structure

In the analysis of phenotypic community structure, using PCA for all data, from all playing surfaces and golf courses, 94% of the variation was accounted for by the first five principal components (PCs). ANOVA for each of these PCs showed significant playing surface treatment differences between samples taken on all golf courses in only PC1 ($P<0.01$) and PC5 ($P<0.01$). Significant differences in phenotypic community were also recorded between golf courses measured on PC1, PC4 and PC5 (Table 1).

Graphical representation of the significant components with respect to playing surfaces shows three distinct groupings of data with surfaces that include USGA rootzone in their construction being negatively loaded on PC1, and the temporary greens being positively loaded on the same axis (Fig. 1a). Fairways and standard tees and greens were neutrally loaded in both axes. Negative weightings in these groups indicates communities dominated by Gram negative bacteria (16:1ω7 c and 16:1ω7 t) and Type I methanotrophs (16:0) (Zelles et al., 1992). PLFA biomarkers found to be prevalent in communities associated with a wide range of bacteria (18:1ω9 t and 19:0 c) (Zelles et al., 1992; Pawlett, 2002) were positively weighted (Fig. 1b). A projection of PC1 and PC4 grouped by golf courses shows three clusters on PC1, Courses A, B and D; Course C and Course E, from negative to positive loadings respectively (Fig. 2a). These groupings are not an artefact of the relationships shown in Fig. 1(a), as there are no similarities in any orientation between different play surface type and golf courses. The weightings attributed to these delineations of community structure are dominated by Gram negative bacteria and Type I methanotrophs (16:1ω7 c, 16:1ω7 t and 16:0, respectively; (Zelles et al., 1992) in the negative weightings and Gram positive bacteria (18:1ω9 t, 18:1ω9 c and 19:0 c) (Zelles, 1999) in positive weightings (Fig. 2b).

PCA and subsequent ANOVA was also carried out on PLFA data from each individual golf course in order to test for differences between playing surfaces at this scale. No significant differences were found between playing surfaces with respect to PC1-3 for Courses A, B or D (Table 2). However, Course C showed significant difference on PC2 ($P = 0.04$). PCA of biomarkers from Course E showed significant differences on PC1 only ($P<0.01$). Post-hoc analysis, using Tukey LSD, showed that USGA greens (UG) were significantly different and almost five times more variable than all other playing surfaces tested at that golf

Table 1
One-way ANOVA of PC 1–5 derived from different surfaces ($n = 6$) and from different courses ($n = 5$)

	% variance	Grouping variable			
		Surface		Course	
		F	*P*	*F*	*P*
PC 1	51	7.08	<0.01	13.57	<0.01
PC 2	12	1.13	0.35	1.98	0.11
PC 3	8.0	2.09	0.08	1.37	0.25
PC 4	7.4	0.66	0.66	2.85	0.03
PC 5	6.2	4.89	<0.01	13.14	<0.01

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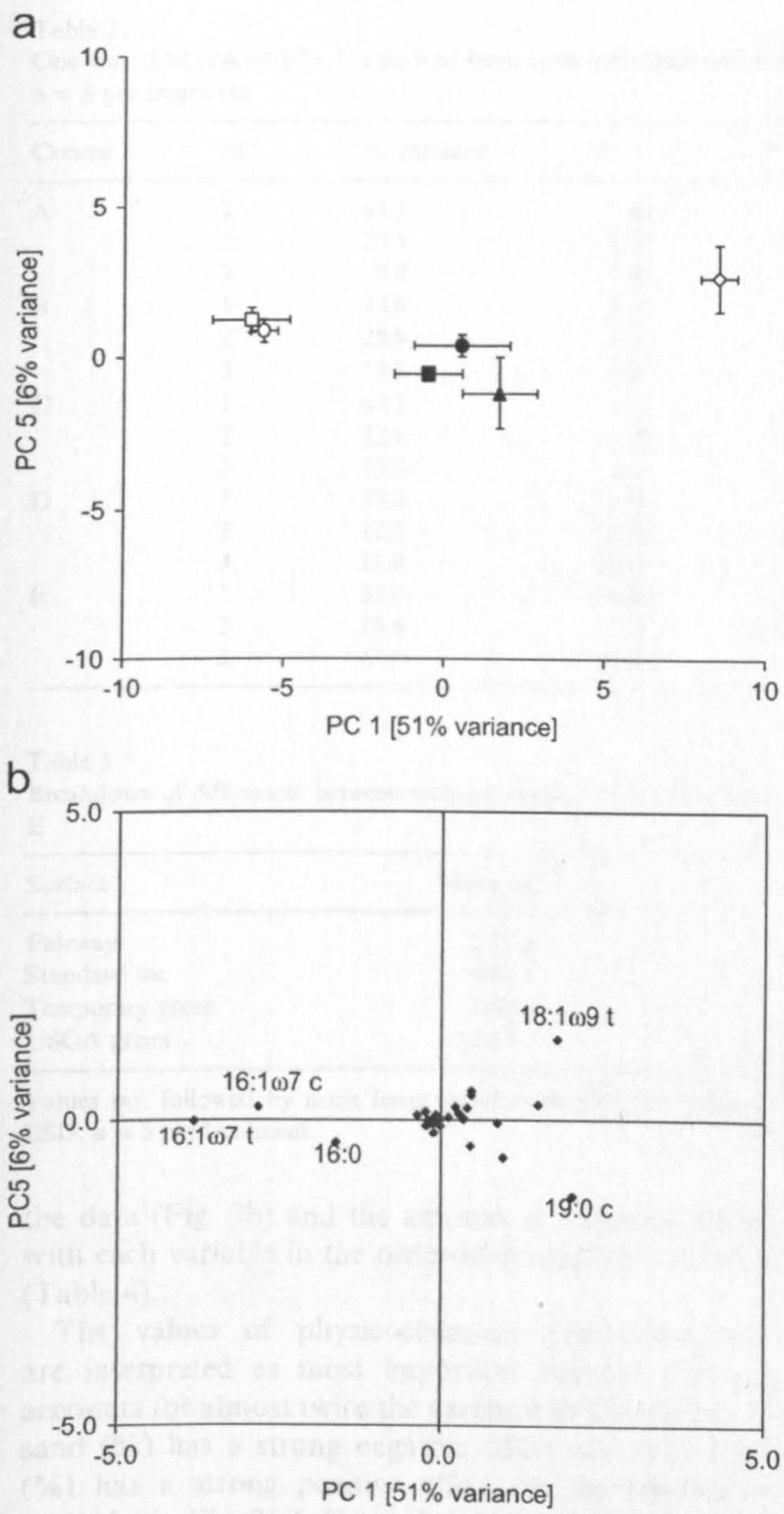

Fig. 1. Principal component (PC) analysis of PLFA data derived from five golf courses: (a) projection of mean co-ordinates of significant PCs with respect to tees (●), USGA topped tees (○), fairways (▲), greens (■), USGA greens (□) and temporary greens (◇) found on golf courses. Whiskers show standard error of the mean; (b) dominantly weighted loading values of PLFAs attributed to significant differences between playing surfaces projected in Fig. 1(a).

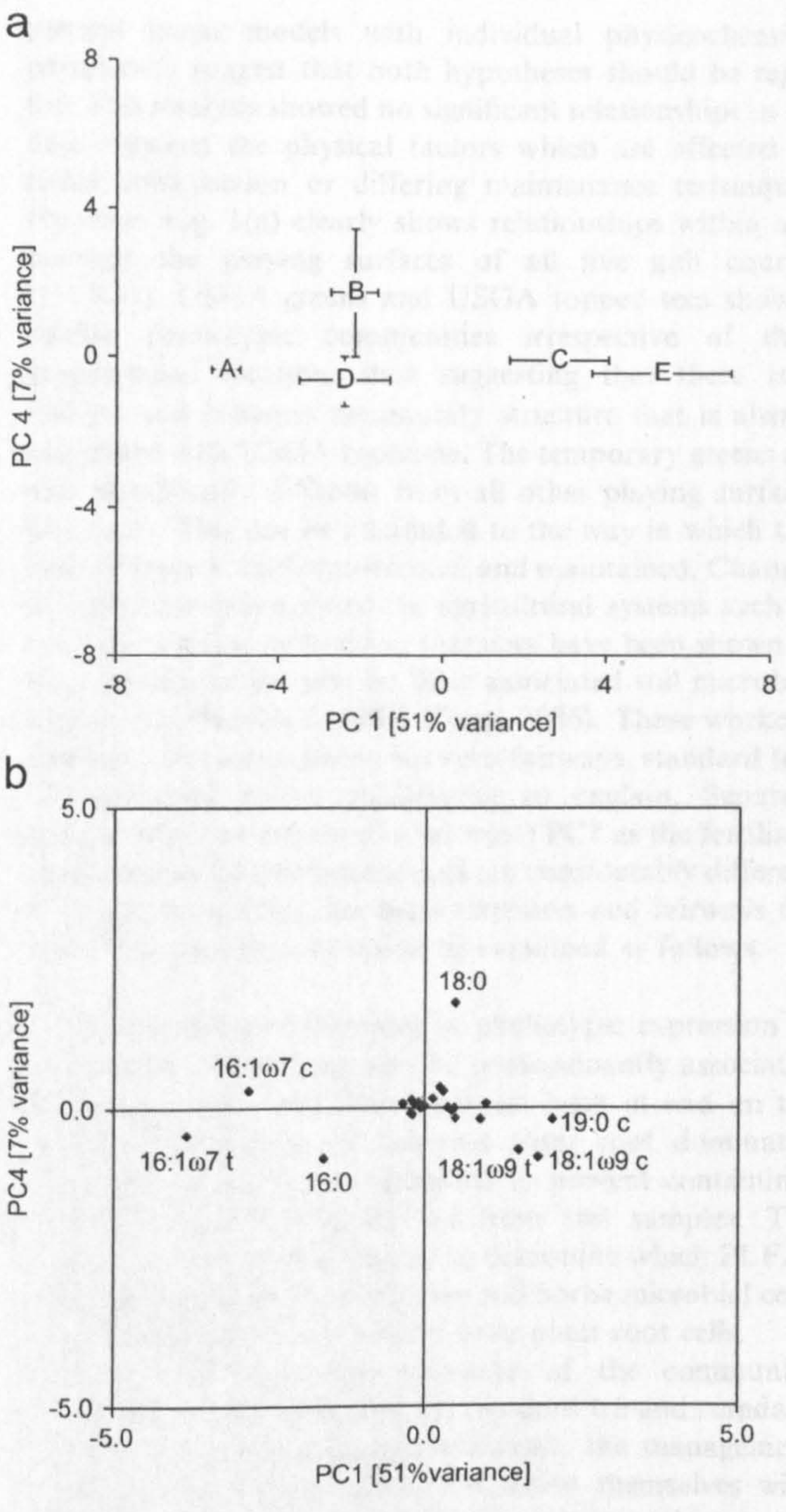

Fig. 2. Principal component (PC) analysis of PLFA data derived from five golf courses: (a) projection of mean co-ordinates of significant PCs with respect to golf course A–E. Whiskers show standard error of the mean; (b) dominantly weighted loading values attributed to significant differences between playing surfaces projected in Fig. 2(a).

course (Table 3). Analysis of relationships between PC1 and physical factors from each play surface measured with general linear models showed poor fits between individual physical factors and the most significant variance associated with the PLFA data ($P>0.05$ in all cases).

3.2. Canonical correlation analysis

When both physical and PLFA variables from all golf courses were subjected to CCA a highly significant relationship was inferred ($P<0.001$, canonical $R=0.96$). A projection of $CR1_{(Physical)}$ vs. $CR1_{(PLFA)}$ demonstrates this (Fig. 3a). Grouping of variables along a straight line indicates significant relationships in this projection. Significant differences were recorded on both $CR1_{(Physical)}$ and $CR1_{(PLFA)}$; $P<0.01$ in both cases. Three distinct clusters were identified using the Tukey honest significant difference: USGA greens and USGA topped tees; fairways and standard tees; temporary greens. Standard greens were intermediate to both USGA topped tees and standard tees. The distribution of these groups can be interpreted by the weightings for CR1 in the orientation in which they affect

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Table 2
One way ANOVA of PCs 1–3 derived from each individual golf course, $n = 5$ per treatment

Course	PC	% variance	F	P
A	1	44.3	0.81	0.47
	2	27.5	1.20	0.34
	3	9.8	3.42	0.07
B	1	43.6	0.32	0.74
	2	25.9	0.57	0.58
	3	14.8	0.29	0.75
C	1	45.3	1.74	0.22
	2	22.6	4.39	0.04
	3	13.3	0.06	0.94
D	1	57.2	0.65	0.54
	2	12.7	0.99	0.40
	3	11.0	0.48	0.63
E	1	55.0	58.23	<0.01
	2	16.6	0.02	>0.99
	3	13.4	0.75	0.54

Table 3
Breakdown of differences between surfaces within PC1 relating to Course E

Surface	Mean PC 1	SE
Fairway	5.13 a	0.35
Standard tee	4.03 a	0.38
Temporary green	3.40 a	0.58
USGA green	−12.6 b	2.06

Values not followed by same letter significantly different using Tukey LSD. $n = 5$ per treatment.

the data (Fig. 3b) and the amount of variance associated with each variable in the order-of-magnitude of this effect (Table 4).

The values of physicochemical component weights are interpreted as most important because $CR1_{(Physical)}$ accounts for almost twice the variance as $CR1_{(PLFA)}$, hence sand (%) has a strong negative effect and total nitrogen (%) has a strong positive effect on the orientation of variables in Fig. 3(a). From the nine variables that account for more than 50% of the variance associated with CR1, two-thirds are physical factors. The most significant PLFA biomarkers were 16:1ω7 c, an indicator of communities dominated by Gram negative bacteria and 17:0 isomers, a eubacterial biomarker (Zelles et al., 1992).

This analysis resolves clear microbiological differences that are dependent upon the physicochemical parameters of the soil at each of the five golf courses (Fig. 4). Each golf course forms a discrete group on both axes ($P<0.01$). These groups reflect spatial relationships between the golf courses, with geographically related golf courses locations being adjacent in this projection.

4. Discussion

PCA and post hoc ANOVA showed significantly different treatment groups, however subsequent univariate general linear models with individual physicochemical parameters suggest that both hypotheses should be rejected. This analysis showed no significant relationships in the data between the physical factors which are affected by either construction or differing maintenance techniques. However Fig. 1(a) clearly shows relationships within and between the playing surfaces of all five golf courses ($P<0.01$). USGA greens and USGA topped tees showed similar phenotypic communities irrespective of their geographical location, thus suggesting that there is a distinct and coherent community structure that is always associated with USGA rootzone. The temporary greens are also significantly different from all other playing surfaces ($P<0.01$). This can be attributed to the way in which this type of green is both constructed and maintained. Changes in vegetation management in agricultural systems such as radically altering cultivation practices have been shown to have significant impacts on their associated soil microbial community (Hedlund, 2002; Clegg, 2006). These workers' findings make associations between fairways, standard tees and standard greens challenging to explain. Separate groups would be expected in (at least) PC1 as the fertiliser, chemical and maintenance inputs are considerably different with greens receiving the most attention and fairways the least. These similarities might be explained as follows:

1. Any significant differences in phenotypic expression of microbial community may be predominantly associated with the roots and thatch present both in and on the soil. This portion of soil and plant root dominated material (thatch) was discarded to prevent contamination of the PLFAs derived from soil samples. The analysis technique is unable to determine which PLFAs in the sample are derived from soil borne microbial cells and those which are derived from plant root cells.
2. While the phenotypic structure of the community associated with each fairway, standard tee and standard green are not significantly different, the management differences could potentially manifest themselves with respect to the community size (a parameter which could not be measured using this method of PLFA analysis, this method reports proportional community structure on a mol% basis). Differences in size and activity of the microbial community were shown by Karp and Nelson (2004) between USGA rootzones and golf course soils.
3. Interactions between the microbial community and the soil in which they live is governed by more factors than can be described using PCA followed by simple linear regressions.

On the basis of the PCA analysis Hypothesis 2 is tentatively rejected because whilst there were statistically significant clusters of data associated with PC1 in Fig. 2(a) ($P<0.01$), no causal relationships with geographical location were evident. This would imply no relationship between the soil microbial community and physicochemical differences measured between playing surfaces. Other

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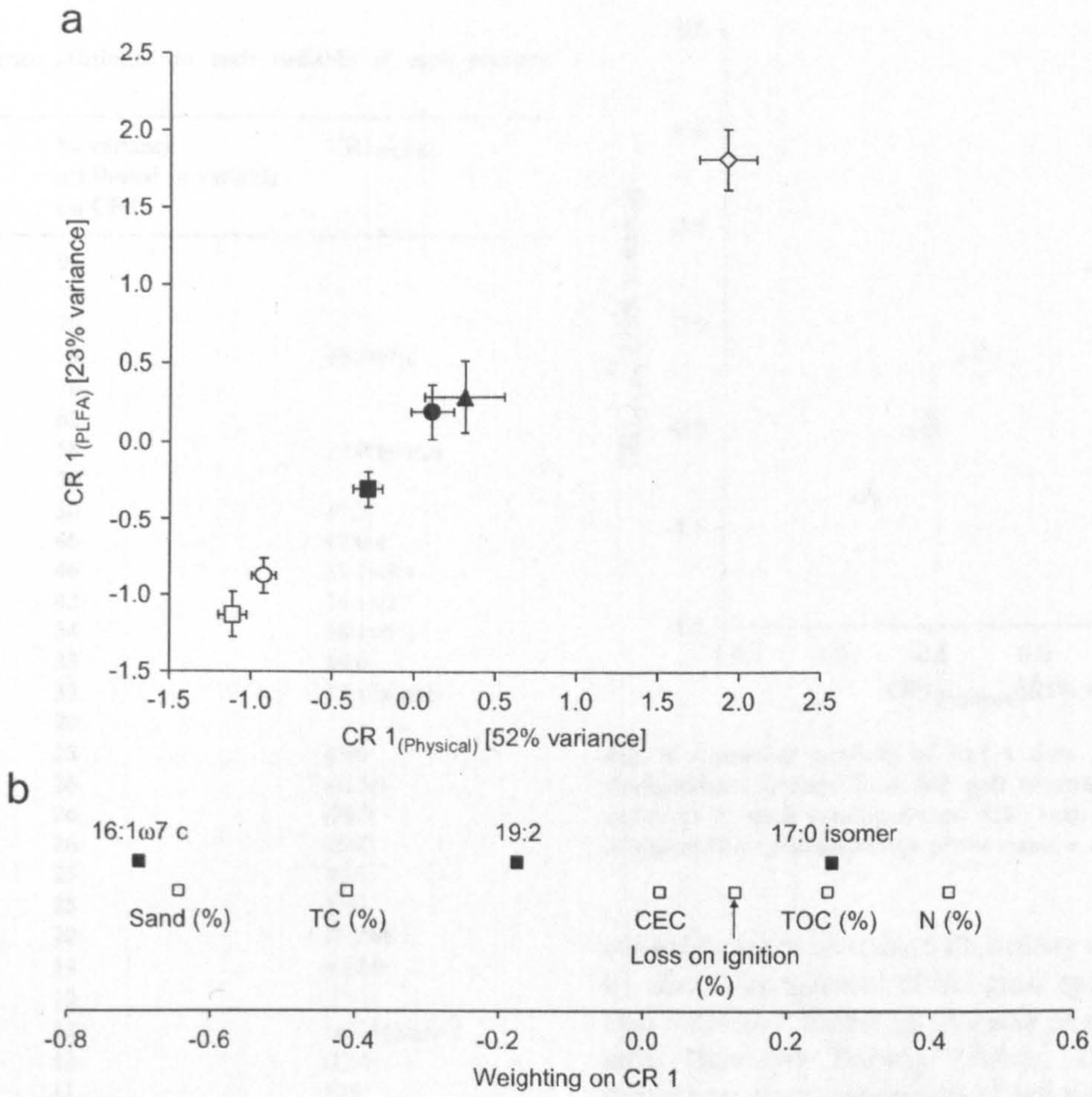

Fig. 3. Canonical correlation analysis of PLFA data and physical soil characteristics based on playing surface type: (a) projection of mean co-ordinates of each canonical root (CR) with respect to standard tee (●), USGA topped tee (○), fairways (▲), standard greens (■), USGA greens (□) and temporary greens (◇) found on golf courses. Whiskers show standard error of the mean; (b) weighting attributed to variables accounting for the top half of the variance from canonical analysis, see Table 4. Closed symbols indicate PLFA variables, open symbols indicate physical variables.

parameters may be responsible for the differences found in PC1.

When data were re-analysed using CCA including all physicochemical variables in the analysis rather than using them as part of a *post-hoc* test, the relationships between phenotypic microbial community structure and physico-chemical soil parameters were resolved and based on this analysis, both Hypotheses 1 and 2 were firmly accepted. All of the playing surfaces containing USGA rootzone group at the negative extreme of this analysis. Sand (%) and 16:1ω7—an indicator of communities dominated by Gram negative bacteria (Zelles et al., 1992)—were responsible for these trends. These findings are broadly in line with those of Karp and Nelson (2004), who conducted a study using agar-enriched colony counting in combination with direct soil DNA extraction and showed that soils collected from golf courses in the USA are dominated by Gram positive bacteria. The USGA rootzone they tested contained principally Gram negative bacteria (Karp and Nelson, 2004). These findings are reflected in results for these UK soils. Steenwerth et al. (2002) have also investigated physico–biological interactions of microbes in pasture and agricultural soils using CCA. They conclude that non-native grasslands are associated with unique microbial communities. This effect is demonstrated here with USGA greens and USGA topped tees being manipulated to maintain a dominant grass community of fescues (*Festuca* spp.) and creeping bents (*Agrostis* spp.) rather than the ecologically successive grasses (*Poa* spp.).

In the positively loaded extreme of this analysis are the temporary greens: the interpretation here is as with the PLFA data alone. The construction and maintenance of these playing surfaces significantly affects the soil microbial community. The loadings seen in Fig. 3(b) confirm the suggested mechanism for these differences, with the weightings being positively drawn by total nitrogen (%) and total organic carbon (%). An imbalance in these two variables has been shown to lead to the development of thatch, measured as percentage weight loss on ignition (Randell et al., 1972; Hope, 1990; Perris, 1996).

The inclusion of these physicochemical factors also resolves differences between fairways, standard tees and

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Table 4
Proportion of variance attributed to each variable of each primary canonical root

$CR1_{(Physical)}$	% variance attributed to variable on CR1	$CR1_{(PLFA)}$
Sand (%)	94	
N (%)	78	
Organic matter (%)	76	
	74	16:1w7 c
TOC (%)	70	
TC (%)	62	
	58	17:0 isomer
CEC	50	
	50	19:2
	46	19:0 c
	46	18:1w9 t
	42	16:1w7 t
	34	18:1w9 c
	33	19:0
	33	17:1 isomer
C:N ratio	30	
	28	17:0
	26	ai15:0
	26	i16:0
	26	16:0
	25	20:0
	25	15:0
	20	18:2w6 c
	19	ai17:0
	18	i17:0
	13	14:1 isomer a
	13	i15:0
	11	15:1
Clay (%)	8.6	
	8.1	Me17:0 isomer
	7.6	18:0
	3.5	14:1 isomer b
pH	3.2	
	2.8	18:1w7 t
	2.6	18:0 isomer
	1.3	18:1 isomer
	1.2	17:0 c
	0.1	16:1 isomer

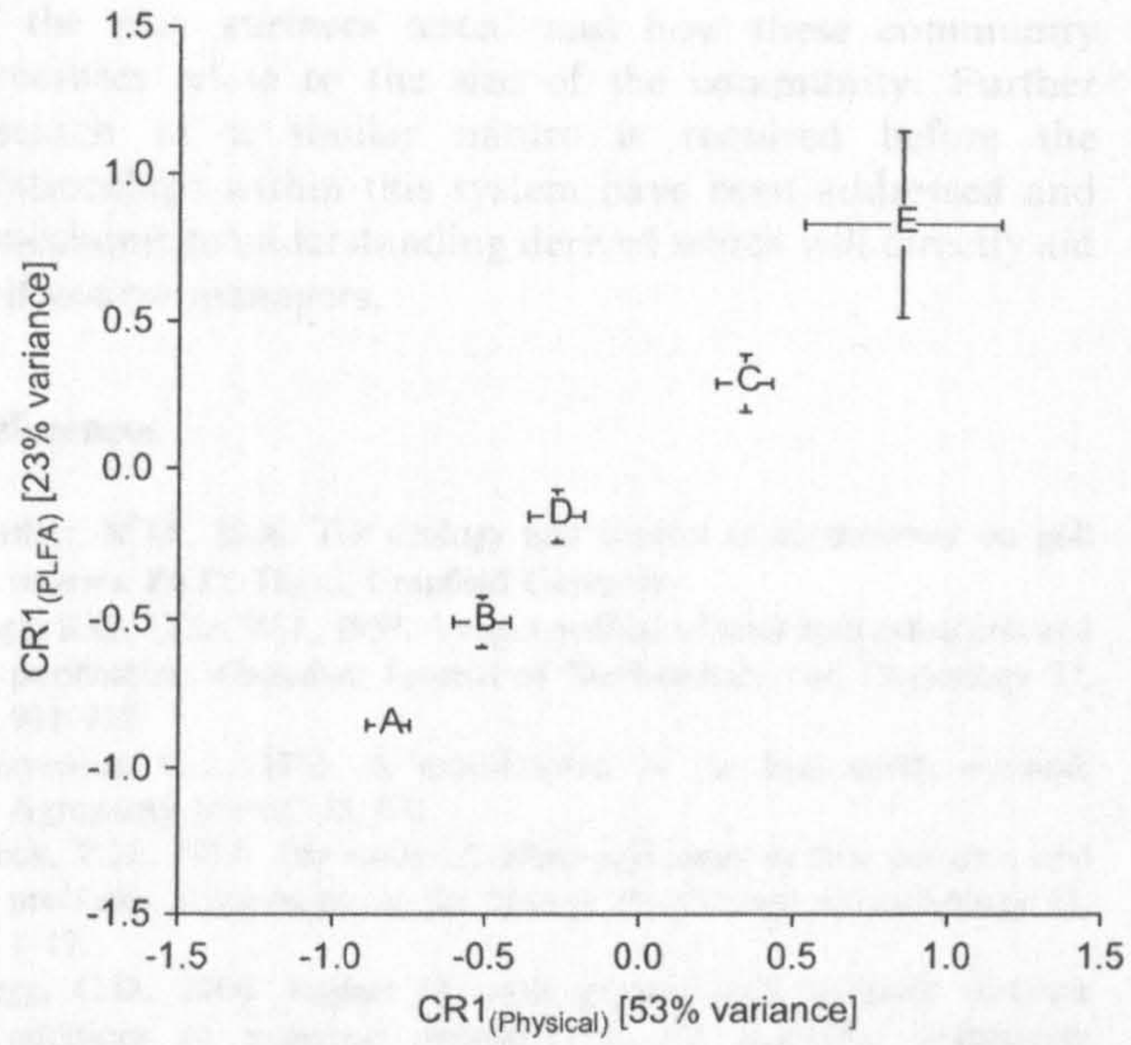

Fig. 4. Canonical analysis of PLFA data and physicochemical soil characteristics derived from five golf courses. Projection of mean co-ordinates of each canonical root (CR) with respect to Courses A–E. Whiskers show standard error of the mean, $n = 15$.

standard greens. This analysis indicates that standard greens are distinct from standard tees and fairways ($P<0.05$). This separation reflects the differences in both chemical and management inputs to these playing surfaces as has been shown in other grasslands (Hedlund, 2002; Clegg, 2006). Management practices such as top dressing with sandy materials in order to improve drainage and soil aeration (Hope, 1990; Perris, 1996) have a clear influence on the phenotypic expression of the soil microbial community. The standard tees and fairways are not significantly different ($P>0.05$) therefore any differences in nutrient input, through the increased frequency of mowing and any other management techniques do not affect the phenotypic structure of the microbial community within the soil. The physical construction of the soil profiles of the fairway and the tee are very similar. The tee is essentially an area of the fairway that has been consolidated to increase bulk density and the turf improved by close management of the grass species present. The tee also receives a higher proportion of player traffic per unit area than the fairway (Perris, 1996). Despite these differences the composition of the nutrient inputs and the physical stresses are roughly equivalent on these two playing surfaces, which may account for the lack of significant difference in phenotypic microbial community structure. Lack of significant differences may also have been due to the level of resolution between samples, which the PLFA analysis method provides. These findings are consistent with those of others where PLFA analysis fails to detect any differences in community structure in pasture lands at different geographical locations and where the time since last tillage operation varies (Steenwerth et al., 2002). The suggested mechanism for this is that the effect that grass growth has on the soil microbial community is rapid, consistent and long-lasting.

The mean variance associated with the physical variables is significantly greater than that with the PLFA variables ($P = 0.03$), this result reflects the composition of the nine individual variables accounting for more than 50% variance on CR1 (Table 4). Two-thirds of these variables are physicochemical measurements with sand (%) dominating the variance within the experiment. This shows that physicochemical factors are the most important in affecting the phenotypic expression of the microbial community within this sand dominated soil. Goodfriend (1998) studied sand dune and spatially related agricultural soils in both Mexico and the USA using the Biolog™ technique to characterise microbial communities. Goodfriend (1998) concluded that the relationships between the microbial

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communities from eight different soils reflect similarities in habitat type more closely than geographical location, which is associated with their phenotypic expression. The anthropogenic soil profile associated with the construction and management of different playing surfaces on geographically separated golf courses are more important in dictating the microbial community structure than the geographical location. Each playing surface associated community identified in Fig. 3(a) is found at one or more golf courses studied in this experiment.

When the physicochemical factors are considered the microbial communities associated with each golf course were significantly different. Golf courses that are geographically close to each other are also proximal in the CCA (Fig. 4), thus Hypothesis 2 is accepted. The increased error associated with the samples from Course E can be attributed to the inclusion of the heavily negatively weighted USGA green samples within this treatment which showed a significant difference at this course (Tables 2 and 3). Each grouping of golf course data points in Fig. 4 contains a combination of at least three playing surfaces; therefore it must be differences in the local environment that are causing this effect. Management by different individuals can be ruled out as an unaccounted for variable as courses C and D (which are managed by the same individual) do not form associations on any axis of the CCA.

Based on these findings it is possible to draw considerably wider conclusions than Goodfriend (1998). Irrespective of variation in geography and individual management strategies for different turf playing surfaces on a golf course, the phenotypic microbial community structure appears to reflect similarities in the physicochemical component of the habitat type to a marked degree. The microbial community associated with two golf greens (or any other golf course playing surface studied here) that have been constructed and managed as per industrial standards (Hope, 1990; Perris, 1996) will be similar. The data also highlights the fact that care must be taken when using the PLFA technique for inter-site analysis. By design the technique is a phenotypic measurement of the soil microbial community. The phenotype of an organism by definition is the tangible properties expressed by the interaction of the genotype and its environment. From these measurements the expression of these phenotypic differences are distinct for the soil microbial community. Where different physicochemical properties are known to exist care must be taken to parameterise the variation associated with the habitat difference before any non-site specific treatment differences are investigated. It is through the understanding of these expressed microbial characteristics that we can begin to understand and therefore manipulate the nutrient cycling and soil structural elements in which these microorganisms play a significant role. Another fundamental question posed by this research is whether there are significant differences in phenotypic microbial community structure at different depths on each of the play surfaces tested and how these community structures relate to the size of the community. Further research of a similar nature is required before the relationships within this system have been addressed and a mechanistic understanding derived which will directly aid golf course managers.

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Appendix II: Quadrat survey and co-variables

Data database structure

A Microsoft Jet 4.0 OLE database using Microsoft Access was constructed to store and analyses the quadrat observations, physicochemical and environmental co-variables that were recorded from surveys described in Chapter 3 (Figure AII.1).

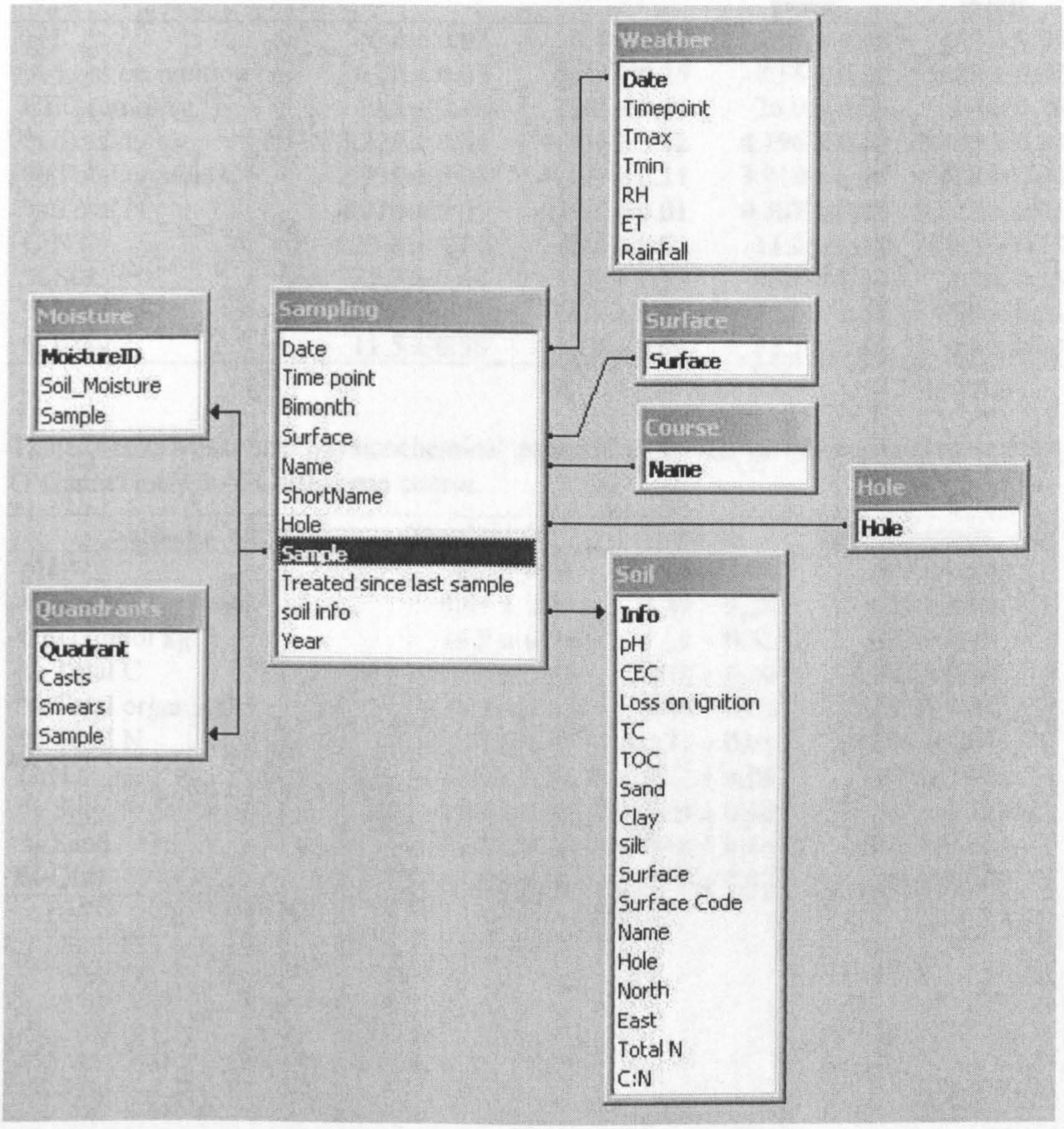

Figure AII.1: Relationships within database constructed to store and query quadrat survey data

Summary of physicochemical co-variables

The following measurements were made of soil physicochemical parameters to be used as co-variables in analysis conducted in Chapters 3 and 6.

Table AII.1: Mean soil physicochemical parameters for all surfaces surveyed at Buckingham golf club.

Surface	Standard tee	Fairway	Temporary green	USGA green
pH	6.6 ± 0.02	6.4 ± 0.04	6.1 ± 0.08	7.3 ± 0.01
% Loss on ignition	6.35 ± 0.07	9.95 ± 0.25	8.56 ± 0.30	0.88 ± 0.02
CEC (cmol kg^{-1})	19.5 ± 0.36	29.2 ± 0.44	26.0 ± 0.66	5.4 ± 0.26
% Total C	3.229 ± 0.04	4.701 ± 0.12	4.196 ± 0.16	0.453 ± 0.01
% Total organic C	2.930 ± 0.03	4.393 ± 0.11	3.910 ± 0.14	0.402 ± 0.01
% Total N	0.276 ± 0.01	0.461 ± 0.01	0.387 ± 0.02	0.023 ± 0.01
C:N	11.8 ± 0.16	10.2 ± 0.05	11.0 ± 0.22	19.5 ± 0.13
% Silt	25.3 ± 0.68	57.1 ± 0.73	46.8 ± 1.44	3.7 ± 0.18
% Sand	62.9 ± 0.53	30.7 ± 0.71	42.2 ± 1.34	95.6 ± 0.21
% Clay	11.5 ± 0.58	12.0 ± 0.51	11.1 ± 0.66	0.8 ± 0.13

Table AII.2: Mean soil physicochemical parameters for all surfaces surveyed at John O'Gaunt Golf Club, Carthagena course.

Surface Code	Standard tee	Fairway	Standard green
pH	6.7 ± 0.01	5.3 ± 0.04	6.7 ± 0.02
% Loss on ignition	4.41 ± 0.05	4.85 ± 0.20	4.77 ± 0.05
CEC (cmol kg^{-1})	14.9 ± 0.51	12.1 ± 0.62	10.3 ± 0.48
% Total C	2.157 ± 0.05	2.038 ± 0.10	2.208 ± 0.04
% Total organic C	2.042 ± 0.05	1.969 ± 0.09	2.137 ± 0.04
% Total N	0.157 ± 0.01	0.171 ± 0.01	0.186 ± 0.01
C:N	12.2 ± 0.04	12.2 ± 0.05	13.3 ± 0.06
% Silt	17.4 ± 0.40	19.9 ± 0.60	9.2 ± 0.25
% Sand	75.7 ± 0.33	70.6 ± 0.66	84.7 ± 0.25
% Clay	7.0 ± 0.22	9.5 ± 0.40	6.1 ± 0.15

Table AII.3: Mean soil physicochemical parameters for all surfaces surveyed at Woburn Golf and Country Club, Dukes course.

Surface Code	Standard tee	Fairway	Standard green
pH	6.7 ± 0.03	5.7 ± 0.03	6.3 ± 0.05
% Loss on ignition	3.53 ± 0.12	4.36 ± 0.07	4.34 ± 0.11
CEC (cmol kg^{-1})	9.1 ± 0.45	12.0 ± 0.30	10.9 ± 0.37
% Total C	1.635 ± 0.04	2.292 ± 0.05	2.746 ± 0.10
% Total organic C	1.571 ± 0.04	2.183 ± 0.05	2.557 ± 0.09
% Total N	0.124 ± 0.01	0.135 ± 0.01	0.183 ± 0.01
C: N	14.2 ± 0.27	17.2 ± 0.15	18.5 ± 0.64
% Silt	7.3 ± 0.25	8.1 ± 0.20	4.5 ± 0.18
% Sand	88.8 ± 0.31	88.1 ± 0.23	91.2 ± 0.21
% Clay	3.8 ± 0.14	4.0 ± 0.12	4.3 ± 0.14

Table AII.4: Mean soil physicochemical parameters for all surfaces surveyed at John O'Gaunt Golf Club, John O'Gaunt course.

Surface Code	Standard tee	Fairway	Standard green
pH	6.8 ± 0.01	5.5 ± 0.03	6.4 ± 0.02
% Loss on ignition	4.92 ± 0.07	10.10 ± 0.50	5.22 ± 0.07
CEC (cmol kg^{-1})	14.8 ± 0.39	20.6 ± 0.68	15.1 ± 0.47
% Total C	2.462 ± 0.04	4.703 ± 0.23	2.735 ± 0.04
% Total organic C	2.329 ± 0.04	4.517 ± 0.22	2.676 ± 0.04
% Total N	0.205 ± 0.01	0.427 ± 0.02	0.229 ± 0.01
C:N	12.0 ± 0.06	11.0 ± 0.06	12.2 ± 0.07
% Silt	21.0 ± 0.37	32.2 ± 1.47	12.6 ± 0.31
% Sand	74.7 ± 0.40	56.6 ± 1.38	81.4 ± 0.38
% Clay	3.8 ± 0.15	11.2 ± 0.33	5.9 ± 0.19

Table AII.5: Mean soil physicochemical parameters for all surfaces surveyed at Woburn Golf and Country Club, Marquess course.

	Marquess		
Surface Code	USGA topped tee	Fairway	USGA green
pH	6.0 ± 0.03	5.9 ± 0.05	6.4 ± 0.01
% Loss on ignition	2.59 ± 0.03	2.12 ± 0.05	2.94 ± 0.04
CEC (cmol kg^{-1})	9.2 ± 0.46	12.2 ± 0.51	12.1 ± 0.53
% Total C	1.036 ± 0.02	0.744 ± 0.04	1.118 ± 0.02
% Total organic C	0.948 ± 0.02	0.707 ± 0.03	1.093 ± 0.02
% Total N	0.041 ± 0.01	0.038 ± 0.01	0.041 ± 0.01
C:N	25.8 ± 0.21	18.9 ± 0.14	28.1 ± 0.21
% Silt	2.6 ± 0.11	5.0 ± 0.15	2.3 ± 0.17
% Sand	90.3 ± 0.22	84.3 ± 0.30	89.5 ± 0.18
% Clay	7.1 ± 0.22	10.8 ± 0.21	8.1 ± 0.11

Summary of environmental co-variables

The following weather variables were recorded at Cranfield University at Silsoe and used a co-variables in analysis conducted in Chapter 3.

Table AII.6: Mean environmental parameters recorded for seven prior to sampling date at Cranfield University at Silsoe.

Date Sampled	Evapotranspiration (mm d^{-1})	Rainfall (mm)	% Relative Humidity	Maximum temperature (°C)	Minimum temperature (°C)
17/06/2004	4.00	0.0	73.0	22.0	12.0
21/06/2004	3.00	2.0	81.0	18.0	8.0
07/07/2004	2.93	3.5	81.0	19.2	9.3
20/07/2004	2.40	2.5	90.4	21.8	13.0
27/07/2004	2.67	0.3	81.3	22.1	12.2
24/08/2004	2.21	4.8	87.9	20.9	12.4
27/08/2004	2.13	2.7	86.6	20.3	12.3
15/09/2004	1.97	1.7	83.3	19.6	10.2
21/09/2004	1.60	0.9	82.7	17.6	8.7
22/09/2004	1.49	0.9	83.7	17.8	9.6
07/10/2004	0.94	4.5	92.6	15.4	7.6
09/11/2004	0.29	1.8	97.1	11.6	7.0
10/11/2004	0.27	1.9	97.1	11.1	6.3
08/12/2004	0.10	0.1	99.4	8.6	3.6
12/01/2005			Missing data		
18/01/2005			Missing data		
23/02/2005	0.57	0.5	86.0	5.2	0.1
08/03/2005	0.60	0.0	81.7	5.8	0.7
28/03/2005	1.04	1.6	92.3	13.7	5.9
28/04/2005	1.64	1.4	85.4	14.6	5.6
26/05/2005	2.33	4.5	86.4	17.7	9.4
24/05/2005	1.94	4.8	87.0	15.8	7.2
29/03/2005	1.06	1.8	92.7	13.0	5.0
20/06/2005	3.10	0.6	81.9	24.4	13.2
07/07/2005	2.17	1.0	89.0	18.7	12.1
21/07/2005	3.65	0.1	72.4	23.5	12.6
16/08/2005	2.23	1.1	84.4	21.7	10.8
22/09/2005	1.31	0.1	89.4	17.7	8.7
27/09/2005	1.34	0.7	88.4	18.8	9.2
12/10/2005	0.83	0.9	92.9	18.7	11.6
17/11/2005	0.27	0.4	92.7	9.8	1.7
24/11/2005	0.19	0.5	97.9	6.0	-2.1
14/12/2005	< 0.01	0.2	98.9	7.0	1.7
20/01/2006	0.30	1.3	96.4	9.5	5.8
24/01/2006	0.34	0.6	95.4	6.8	0.7
25/01/2006	0.33	0.2	95.1	6.1	-0.3
21/02/2006	0.56	0.5	93.7	7.5	2.4

Table AII.6 cont.: Mean environmental parameters recorded for seven prior to sampling date at Cranfield University at Silsoe

Date Sampled	Evapotranspiration (mm d^{-1})	Rainfall (mm)	% Relative Humidity	Maximum temperature (°C)	Minimum temperature (°C)
23/03/2006	1.03	0.1	79.7	6.2	0.6
25/04/2006	1.50	0.4	93.9	14.7	7.7
09/05/2006	2.20	2.1	89.0	18.9	9.7
17/05/2006	2.28	0.2	88.7	19.4	7.9

Appendix III: Mustard extraction surveys

The earthworms of the following species were recovered during the calibration of the mustard extraction technique, Chapter 4.

Table AIII:1: Mean of each species earthworms recovered per treatment in mustard extraction calibration trials. n = 7 per treatment.

Treatment	Water			Control			Mustard		
Recovery method	Handsorted	Surface extraction	Total	Handsorted	Surface extraction	Total	Handsorted	Surface extraction	Total
Allolobophora chlorotica	5.7 ± 1.8	-	5.7 ± 1.8	6.4 ± 1.4	-	6.4 ± 1.4	3.3 ± 1.2	2.0 ± 1.0	5.3 ± 1.4
Aporrectodea calagonsis	7.1 ± 2.9	-	7.1 ± 2.9	12.3 ± 2.6	-	12.3 ± 2.6	3.7 ± 2.4	1.1 ± 1.0	4.9 ± 3.1
Aporrectodea rosea	18.3 ± 4.2	-	18.3 ± 4.2	26.3 ± 2.1	-	26.3 ± 2.1	16.1 ± 3.4	7.6 ± 1.3	23.7 ± 3.6
Lumbricus castaneus	3.1 ± 0.4	-	3.1 ± 0.4	7.9 ± 2.2	-	7.9 ± 2.2	2.6 ± 0.9	1.6 ± 0.6	4.1 ± 0.7
Lumbricus festivus	0.0	-	0.0	0.6 ± 0.4	-	0.6 ± 0.4	0.1 ± 0.1	0.0	0.1 ± 0.1
Lumbricus rubellus	4.1 ± 1.8	-	4.1 ± 1.8	5.9 ± 1.1	-	5.9 ± 1.1	1.1 ± 0.4	2.0 ± 0.9	3.1 ± 0.9
Lumbricus terrestris	3.9 ± 0.7	-	3.9 ± 0.7	6.9 ± 1.2	-	6.9 ± 1.2	3.4 ± 1.1	2.7 ± 0.4	6.1 ± 1.1
Unidentifiable juveniles	0.7 ± 0.3	-	0.7 ± 0.3	1.7 ± 0.7	-	1.7 ± 0.7	0.9 ± 0.6	0.4 ± 0.4	1.3 ± 0.6
Total	43.0 ± 9.1	-	43.0 ± 9.1	67.9 ± 7.6	-	67.9 ± 7.6	31.3 ± 8.6	17.4 ± 2.5	48.7 ± 9.3

The following earthworm species were recovered from golf course fairways between October 2005 and July 2006 (Chapter 5).

Table AIII.2: Mean of each species of earthworm recovered from golf courses fairways surveyed using mustard extraction during October 2005. n= 9 per course.

	Course				
Species	Buckingham	John O'Gaunt	Carthagena	Dukes	Marquess
Allolobophora chlorotica	0.00	0.00	0.00	0.00	0.11 ± 0.03
Aporrectodea calagonsis	0.00	0.00	0.00	0.00	0.00
Aporrectodea longa	0.14 ± 0.01	0.00	0.09 ± 0.04	0.11 ± 0.01	0.27 ± 0.15
Aporrectodea rosea	0.33 ± 0.14	0.26 ± 0.10	0.44 ± 0.14	0.39 ± 0.13	0.37 ± 0.16
Lumbricus festivus	0.00	0.00	0.00	0.00	0.00
Lumbricus rubellus	0.01 ± 0.01	0.23 ± 0.07	0.07 ± 0.5	0.03 ± 0.01	0.03 ± 0.01
Lumbricus terrestris	0.24 ± 0.14	0.12 ± 0.01	0.06 ± 0.04	0.26 ± 0.14	0.01 ± 0.01
Unidentifiable juveniles	0.00	0.00	0.00	0.00	0.00

Table AIII.3: Mean of each species of earthworm recovered from golf courses fairways surveyed using mustard extraction during January 2006. n= 9 per course.

	Course				
Species	Buckingham	John O'Gaunt	Carthagena	Dukes	Marquess
Allolobophora chlorotica	0.00	0.00	0.00	Missing data	Missing data
Aporrectodea calagonsis	0.00	0.01 ± < 0.01	0.00	Missing data	Missing data
Aporrectodea longa	0.08 ± 0.04	0.11 ± 0.01	0.09 ± 0.04	Missing data	Missing data
Aporrectodea rosea	0.13 ± 0.04	0.28 ± 0.10	0.14 ± 0.08	Missing data	Missing data
Lumbricus festivus	0.00	0.00	0.00	Missing data	Missing data
Lumbricus rubellus	0.07 ± 0.03	0.13 ± 0.07	0.20 ± 0.01	Missing data	Missing data
Lumbricus terrestris	0.04 ± 0.03	0.04 ± 0.02	0.02 ± 0.01	Missing data	Missing data
Unidentifiable juveniles	0.00	0.00	0.00	Missing data	Missing data

Table AIII.4: Mean of each species of earthworm recovered from golf courses fairways surveyed using mustard extraction during April 2006. n= 9 per course.

	Course				
Species	Buckingham	John O'Gaunt	Carthagena	Dukes	Marquess
Allolobophora chlorotica	0.00	0.00	0.00	0.00	0.00
Aporrectodea calagonsis	0.00	0.00	0.00	0.00	0.00
Aporrectodea longa	0.03 ± 0.01	0.33 ± 0.14	0.03 ± 0.02	0.00	0.32 ± 0.11
Aporrectodea rosea	0.06 ± 0.02	0.01 ± 0.01	0.27 ± 0.08	0.00	0.01 ± 0.01
Lumbricus festivus	0.00	0.00	0.00	0.00	0.00
Lumbricus rubellus	0.13 ± 0.03	0.03 ± 0.02	0.08 ± 0.05	0.12 ± 0.01	0.04 ± .02
Lumbricus terrestris	0.15 ± 0.04	0.11 ± 0.01	0.06 ± 0.03	0.25 ± 0.14	0.13 ± 0.01
Unidentifiable juveniles	0.02 ± 0.01	0.00	0.00	0.16 ± 0.09	0.00

Table AIII.5: Mean of each species of earthworm recovered from golf courses fairways surveyed using mustard extraction during July 2006. n= 9 per course.

	Course				
Species	Buckingham	John O'Gaunt	Carthagena	Dukes	Marquess
Allolobophora chlorotica	0.04 ± 0.02	0.03 ± 0.01	0.00	0.00	0.00
Aporrectodea calagonsis	0.06 ± 0.03	0.05 ± 0.03	0.00	0.00	0.00
Aporrectodea longa	0.05 ± 0.03	0.02 ± 0.02	0.00	0.00	0.03 ± 0.02
Aporrectodea rosea	0.07 ± 0.04	0.03 ± 0.01	0.03 ± 0.01	0.00	0.03 ± 0.01
Lumbricus festivus	0.00	0.00	0.00	0.03 ± 0.03	0.00
Lumbricus rubellus	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.00	0.00
Lumbricus terrestris	0.00	0.01 ± 0.01	< 0.01± 0.03	0.11 ± 0.01	0.00
Unidentifiable juveniles	0.00	0.00	0.00	0.03 ± 0.02	0.00

Table AIII.6: Mean microbial biomass carbon at each month surveyed at each golf course. n=9 per course.

Course	Month	Microbial biomass carbon ($\mu g\ g^{-1}$)
John O'Gaunt	October 2005	749.4 ± 99.4
John O'Gaunt	January 2006	602.7± 125.6
John O'Gaunt	April 2006	437.8 ± 133.9
John O'Gaunt	July 2006	497.4 ± 135.5
Carthagena	October 2005	229.2 ± 28.0
Carthagena	January 2006	291.2 ± 18.5
Carthagena	April 2006	337.5 ± 79.2
Carthagena	July 2006	404.0 ± 65.6
Marquess	October 2005	130.9 ± 31.5
Marquess	January 2006	Missing data
Marquess	April 2006	154.5± 19.0
Marquess	July 2006	143.6 ± 45.1
Dukes	October 2005	271.7± 25.0
Dukes	January 2006	305.8± 37.1
Dukes	April 2006	432.9± 109.6
Dukes	July 2006	219.7 ± 27.8
Buckingham	October 2005	821.9 ± 94.1
Buckingham	January 2006	736.7± 103.8
Buckingham	April 2006	1578.2 ± 186.2
Buckingham	July 2006	972.8 ± 84.1

Appendix IV: Microbial analysis of golf courses

Inter-course analysis

The following PLFA biomarkers were isolated using gas chromatography from samples taken from play surfaces at the five golf courses used throughout this thesis, March 2005 and used in PCA and CCA analysis (Chapter 6).

Table AIV.1: Mean of each PLFA biomarker, recorded as mol % from surfaces surveyed at Buckingham golf courses, March 2005, n=5 per surface.

Biomarker	Surface			
	Standard tee	Fairway	Temporary green	USGA green
14:0	1.16 ± 0.05	1.09 ± 0.06	1.15 ± 0.06	1.46 ± 0.10
14:1 isomer a	1.08 ± 0.06	0.87 ± 0.09	0.94 ± 0.07	1.32 ± 0.10
14:1 isomer b	0.97 ± 0.04	0.83 ± 0.07	0.91 ± 0.03	1.45 ± 0.10
i15:0	6.60 ± 0.39	6.10 ± 0.24	5.13 ± 1.17	3.53 ± 0.32
ai15:0	5.98 ± 0.61	6.16 ± 0.55	5.90 ± 0.67	3.88 ± 0.32
15:1	0.70 ± 0.03	0.64 ± 0.06	1.53 ± 0.93	1.49 ± 0.11
15:0	1.09 ± 0.06	1.07 ± 0.06	0.97 ± 0.15	1.47 ± 0.13
16:1 isomer	1.31 ± 0.08	1.42 ± 0.03	1.23 ± 0.18	1.16 ± 0.08
i16:0	3.62 ± 0.28	4.58 ± 0.08	3.76 ± 0.84	2.35 ± 0.18
16:1ω7 c	6.79 ± 0.33	5.85 ± 0.36	5.60 ± 0.82	14.69 ± 2.07
16:1ω7 t	0.84 ± 0.03	0.92 ± 0.09	2.07 ± 1.25	8.79 ± 1.71
16:0	0.94 ± 0.11	0.85 ± 0.06	1.07 ± 0.15	7.22 ± 3.45
Me17:0 isomer	2.12 ± 0.42	1.60 ± 0.14	2.01 ± 0.36	1.53 ± 0.11
i17:0	3.68 ± 0.77	2.73 ± 0.13	3.51 ± 0.74	1.98 ± 0.08
ai17:0	3.88 ± 0.60	3.15 ± 0.20	3.88 ± 0.78	2.50 ± 0.13
17:0 c	3.19 ± 0.21	3.16 ± 0.16	3.01 ± 0.22	2.77 ± 0.13
17:1 isomer	1.02 ± 0.07	1.15 ± 0.10	0.91 ± 0.07	1.57 ± 0.12
17:0	1.00 ± 0.04	1.06 ± 0.09	1.00 ± 0.06	1.30 ± 0.08
17:0 isomer	3.02 ± 0.23	3.13 ± 0.15	2.79 ± 0.21	1.53 ± 0.10
18:0 isomer	1.19 ± 0.06	1.66 ± 0.11	1.54 ± 0.13	1.53 ± 0.10
18:2ω6 c	4.78 ± 0.73	4.46 ± 0.60	4.05 ± 0.43	3.20 ± 0.32
18:1ω9 c	9.70 ± 0.52	10.46 ± 0.67	9.93 ± 0.71	8.65 ± 1.790
18:1ω9 t	13.46 ± 0.53	13.05 ± 0.51	12.73 ± 1.42	6.90 ± 0.58
18:1ω7 t	1.18 ± 0.02	1.25 ± 0.11	1.21 ± 0.14	1.74 ± 0.16
18:1 isomer	1.89 ± 0.16	2.22 ± 0.21	1.96 ± 0.19	2.42 ± 0.25
18:0	3.55 ± 0.41	3.20 ± 0.34	6.46 ± 2.77	3.34 ± 0.50
19:2	2.94 ± 0.41	3.33 ± 0.11	2.96 ± 0.10	1.97 ± 0.15
19:0 c	9.28 ± 0.55	11.14 ± 0.82	8.87 ± 0.88	3.07 ± 0.22
19:0	1.03 ± 0.15	1.02 ± 0.10	0.90 ± 0.06	2.39 ± 0.21
20:0	1.95 ± 0.22	1.88 ± 0.09	2.02 ± 0.20	2.79 ± 0.37

Table AIV.2: Mean of each PLFA biomarker, recorded as mol % from surfaces surveyed at John O'Gaunt Golf Club, Carthagena course, March 2005, n=5 per surface.

	Surface		
Biomarker	Standard tee	Fairway	Standard green
14:0	1.22 ± 0.05	1.25 ± 0.09	1.50 ± 0.14
14:1 isomer a	1.14 ± 0.04	0.98 ± 0.08	1.49 ± 0.12
14:1 isomer b	1.19 ± 0.06	1.12 ± 0.06	1.15 ± 0.12
i15:0	5.37 ± 0.53	5.54 ± 0.13	6.03 ± 0.46
ai15:0	4.74 ± 0.38	4.63 ± 0.09	5.44 ± 0.28
15:1	1.34 ± 0.12	1.24 ± 0.10	1.43 ± 0.12
15:0	1.52 ± 0.04	1.48 ± 0.12	1.55 ± 0.12
16:1 isomer	1.28 ± 0.10	1.38 ± 0.19	1.21 ± 0.08
i16:0	2.96 ± 0.22	3.56 ± 0.17	3.53 ± 0.27
16:1ω7 c	12.75 ± 0.25	10.45 ± 0.44	11.64 ± 1.33
16:1ω7 t	10.93 ± 2.87	7.79 ± 0.81	8.63 ± 2.70
16:0	3.87 ± 0.97	2.95 ± 0.73	2.89 ± 0.70
Me17:0 isomer	1.40 ± 0.09	1.49 ± 0.17	1.51 ± 0.14
i17:0	2.69 ± 0.08	2.70 ± 0.14	3.15 ± 0.15
ai17:0	2.87 ± 0.07	2.85 ± 0.11	3.19 ± 0.19
17:0 c	3.39 ± 0.29	3.43 ± 0.19	4.79 ± 0.21
17:1 isomer	1.48 ± 0.12	1.51 ± 0.11	1.35 ± 0.16
17:0	1.37 ± 0.04	1.46 ± 0.09	1.41 ± 0.14
17:0 isomer	1.47 ± 0.11	1.56 ± 0.14	1.38 ± 0.19
18:0 isomer	2.03 ± 0.28	1.80 ± 0.16	1.67 ± 0.22
18:2ω6 c	2.43 ± 0.10	2.66 ± 0.36	2.15 ± 0.29
18:1ω9 c	6.14 ± 0.41	7.09 ± 0.94	6.71 ± 0.31
18:1ω9 t	9.14 ± 0.79	9.35 ± 1.05	9.32 ± 0.19
18:1ω7 t	1.34 ± 0.09	3.03 ± 1.59	1.46 ± 0.10
18:1 isomer	1.97 ± 0.13	1.90 ± 0.20	1.53 ± 0.13
18:0	2.66 ± 0.59	2.82 ± 0.54	1.90 ± 0.79
19:2	2.03 ± 0.11	2.28 ± 0.17	1.85 ± 0.17
19:0 c	5.88 ± 0.20	8.20 ± 0.84	6.66 ± 0.22
19:0	1.25 ± 0.11	1.12 ± 0.06	1.22 ± 0.21
20:0	2.15 ± 0.16	2.39 ± 0.16	2.25 ± 0.25

Table AIV.3: Mean of each PLFA biomarker, recorded as mol % from surfaces surveyed at Woburn Golf Club and Country club, Dukes course, March 2005, n=5 per surface.

	Surface		
Biomarker	Standard tee	Fairway	Standard green
14:0	1.30 ± 0.05	1.33 ± 0.09	1.55 ± 0.10
14:1 isomer a	1.12 ± 0.05	1.07± 0.09	1.40 ± 0.16
14:1 isomer b	1.10 ± 0.10	0.91 ± 0.06	1.20 ± 0.17
i15:0	5.68 ± 0.26	5.07 ± 0.32	4.96 ± 0.62
ai15:0	4.78 ± 0.25	3.96 ± 0.27	4.49 ± 0.38
15:1	1.26 ± 0.07	1.35 ± 0.05	1.48 ± 0.17
15:0	1.60 ± 0.10	1.47 ± 0.08	1.62 ± 0.13
16:1 isomer	1.30 ± 0.07	1.39 ± 0.08	1.26 ± 0.07
i16:0	3.28 ± 0.24	3.31 ± 0.19	2.79 ± 0.18
16:1ω7 c	13.33 ± 0.48	11.67 ± 0.33	12.66 ± 0.54
16:1ω7 t	6.80 ± 0.50	7.64 ± 0.37	7.52 ± 0.85
16:0	3.36 ± 0.70	4.06 ± 0.50	3.77 ± 0.96
Me17:0 isomer	1.49 ± 0.03	1.85 ± 0.06	1.50 ± 0.08
i17:0	2.70 ± 0.03	2.68 ± 0.03	2.56 ± 0.17
ai17:0	2.96 ± 0.09	2.93 ± 0.07	2.88 ± 0.11
17:0 c	3.91 ± 0.23	3.32 ± 0.10	3.48 ± 0.20
17:1 isomer	1.38 ± 0.03	1.59 ± 0.05	1.47 ± 0.12
17:0	1.46 ± 0.02	1.53 ± 0.06	1.49 ± 0.08
17:0 isomer	1.54 ± 0.03	1.55 ± 0.21	1.66 ± 0.19
18:0 isomer	1.68 ± 0.04	1.99 ± 0.10	1.76 ± 0.17
18:2ω6 c	2.46 ± 0.30	2.41 ± 0.07	2.42 ± 0.06
18:1ω9 c	6.54 ± 0.34	6.42 ± 0.32	6.11 ± 0.54
18:1ω9 t	8.86 ± 0.37	7.18 ± 0.50	7.81 ± 0.77
18:1ω7 t	1.83 ± 0.09	1.69 ± 0.07	1.83 ± 0.08
18:1 isomer	1.94 ± 0.05	1.98 ± 0.04	1.82 ± 0.09
18:0	3.90 ± 0.17	4.70 ± 0.80	5.14 ± 1.13
19:2	2.21 ± 0.06	2.37 ± 0.10	2.06 ± 0.15
19:0 c	6.66 ± 0.39	8.15 ± 0.51	7.29 ± 0.69
19:0	1.20 ± 0.05	1.49 ± 0.07	1.47 ± 0.19
20:0	2.35 ± 0.11	2.93 ± 0.18	2.55 ± 0.16

Table AIV.4: Mean of each PLFA biomarker, recorded as mol % from surfaces surveyed at John O'Gaunt Golf Club, John O'Gaunt course, March 2005, n=5 per surface.

	Surface		
Biomarker	Standard tee	Fairway	Standard green
14:0	1.69 ± 0.16	1.67 ± 0.18	2.23 ± 0.19
14:1 isomer a	1.22 ± 0.18	1.24 ± 0.23	1.82 ± 0.27
14:1 isomer b	2.25 ± 0.67	0.84 ± 0.05	2.29 ± 1.37
i15:0	6.84 ± 1.04	7.52 ± 0.31	8.55 ± 0.70
ai15:0	5.06 ± 1.19	5.81 ± 0.37	6.03 ± 1.22
15:1	1.14 ± 0.14	0.88 ± 0.06	1.19 ± 0.26
15:0	1.50 ± 0.28	1.41 ± 0.12	1.60 ± 0.10
16:1 isomer	1.59 ± 0.33	1.16 ± 0.10	1.64 ± 0.52
i16:0	4.72 ± 0.87	4.58 ± 0.23	3.91 ± 0.29
16:1ω7 c	9.77 ± 0.56	8.66 ± 0.64	9.20 ± 1.61
16:1ω7 t	6.45 ± 3.41	3.11 ± 0.47	3.73 ± 0.77
16:0	5.20 ± 2.15	1.38 ± 0.31	2.45 ± 0.42
Me17:0 isomer	1.47 ± 0.13	1.78 ± 0.07	1.58 ± 0.13
i17:0	2.70 ± 0.19	2.67 ± 0.17	3.22 ± 0.11
ai17:0	2.87 ± 0.28	2.67 ± 0.21	2.99 ± 0.17
17:0 c	3.19 ± 0.30	2.95 ± 0.35	4.47 ± 01.5
17:1 isomer	1.34 ± 0.21	1.49 ± 0.16	1.16 ± 0.18
17:0	1.17 ± 0.17	1.03 ± 0.16	1.36 ± 0.17
17:0 isomer	2.01 ± 0.26	1.97 ± 0.18	2.04 ± 0.18
18:0 isomer	1.65 ± 0.24	2.12 ± 0.12	1.42 ± 0.12
18:2ω6 c	2.27 ± 0.14	2.65 ± 0.18	2.20 ± 0.24
18:1ω9 c	6.37 ± 0.37	8.75 ± 0.71	7.01 ± 0.37
18:1ω9 t	8.86 ± 1.08	8.17 ± 0.32	9.09 ± 0.57
18:1ω7 t	1.41 ± 0.26	1.89 ± 0.14	2.10 ± 0.24
18:1 isomer	1.95 ± 0.22	1.98 ± 0.23	1.67 ± 0.08
18:0	2.33 ± 0.36	2.70 ± 0.53	2.78 ± 0.47
19:2	2.49 ± 0.28	2.95 ± 0.22	2.07 ± 0.08
19:0 c	6.83 ± 0.55	12.35 ± 1.80	6.92 ± 0.34
19:0	1.33 ± 0.19	1.16 ± 0.07	1.08 ± 0.15
20:0	2.32 ± 0.34	2.45 ± 0.13	2.18 ± 0.27

Table AIV.5: Mean of each PLFA biomarker, recorded as mol % from surfaces surveyed at Woburn Golf Club and Country club, Marquess course, March 2005, n=5 per surface.

	Surface		
Biomarker	USGA topped tee	Fairway	USGA green
14:0	1.38 ± 0.11	1.45 ± 0.08	1.30 ± 0.12
14:1 isomer a	1.35 ± 0.09	1.38 ± 0.08	1.40 ± 0.15
14:1 isomer b	1.46 ± 0.13	1.43 ± 0.23	1.36 ± 0.14
i15:0	4.12 ± 0.23	4.41 ± 0.26	4.23 ± 0.17
ai15:0	4.06 ± 0.18	4.05 ± 0.25	4.21 ± 0.13
15:1	1.56 ± 0.14	1.54 ± 0.05	1.58 ± 0.16
15:0	1.55 ± 0.12	1.46 ± 0.03	1.44 ± 0.11
16:1 isomer	1.34 ± 0.10	1.45 ± 0.02	1.27 ± 0.14
i16:0	2.64 ± 0.17	2.95 ± 0.23	2.65 ± 0.11
16:1ω7 c	13.97 ± 0.42	13.46 ± 0.68	13.99 ± 0.33
16:1ω7 t	8.48 ± 0.44	8.02 ± 0.13	9.19 ± 0.72
16:0	6.04 ± 0.87	5.33 ± 0.92	4.86 ± 0.77
Me17:0 isomer	1.61 ± 0.03	1.54 ± 0.05	1.62 ± 0.07
i17:0	2.44 ± 0.04	2.41 ± 0.10	2.49 ± 0.04
ai17:0	2.86 ± 0.07	3.00 ± 0.06	2.85 ± 0.11
17:0 c	3.49 ± 0.13	3.16 ± 0.24	3.20 ± 0.12
17:1 isomer	1.75 ± 0.06	1.64 ± 0.05	1.63 ± 0.06
17:0	1.54 ± 0.09	1.57 ± 0.12	1.43 ± 0.06
17:0 isomer	1.79 ± 0.06	1.71 ± 0.03	1.71 ± 0.06
18:0 isomer	1.81 ± 0.06	2.36 ± 0.63	1.87 ± 0.17
18:2ω6 c	2.86 ± 0.03	3.74 ± 0.34	2.72 ± 0.19
18:1ω9 c	5.50 ± 0.12	5.76 ± 0.21	5.89 ± 0.41
18:1ω9 t	8.18 ± 0.26	7.60 ± 1.47	8.33 ± 0.60
18:1ω7 t	1.79 ± 0.09	1.83 ± 0.11	1.71 ± 0.09
18:1 isomer	2.39 ± 0.08	2.67 ± 0.27	2.18 ± 0.10
18:0	2.24 ± 0.10	2.08 ± 0.08	2.33 ± 0.15
19:2	2.12 ± 0.06	2.32 ± 0.18	2.25 ± 0.13
19:0 c	4.71 ± 0.29	4.86 ± 0.50	5.38 ± 0.45
19:0	2.27 ± 0.38	2.09 ± 0.38	2.24 ± 0.33
20:0	2.68 ± 0.15	2.76 ± 0.42	2.70 ± 0.11

Intra-course analysis

The following PLFA biomarkers were isolated using gas chromatography from samples taken from John O'Gaunt Golf Club, John O'Gaunt course, March 2006 and used in PCA carried out in Chapter 7.

Table AIV.6: Mean of each PLFA biomarker, recorded as mol % from each depth band on tees at John O'Gaunt Golf Club, John O'Gaunt course. Surveyed in March 2006, n=5 per surface.

	Depth			
Biomarker	0 – 75 mm	76 – 150 mm	151 – 225 mm	0 – 225 mm
14:0	0.80 ± 0.20	0.94 ± 0.18	0.90 ± 0.21	0.93 ± 0.11
14:1 isomer a	1.09 ± 0.22	1.09 ± 0.24	1.07 ± 0.18	1.25 ± 0.12
14:1 isomer b	0.98 ± 0.15	0.94 ± 0.18	1.00 ± 0.18	0.96 ± 0.16
i15:0	3.47 ± 0.72	2.81 ± 0.56	2.89 ± 0.53	2.85 ± 0.31
ai15:0	2.66 ± 0.38	2.43 ± 0.41	2.65 ± 0.52	2.57 ± 0.21
15:1	1.09 ± 0.24	1.52 ± 0.22	1.54 ± 0.20	1.33 ± 0.15
15:0	1.02 ± 0.14	1.08 ± 0.23	0.98 ± 0.16	1.06 ± 0.11
16:1 isomer	0.60 ± 0.18	1.21 ± 0.15	1.27 ± 0.15	1.22 ± 0.12
i16:0	2.40 ± 0.14	2.48 ± 0.29	2.93 ± 0.54	2.19 ± 0.19
16:1ω7 c	5.98 ± 0.71	4.39 ± 0.55	5.14 ± 0.42	5.46 ± 0.22
16:1ω7 t	1.05 ± 0.16	1.44 ± 0.16	1.76 ± 0.30	1.89 ± 0.49
16:0	11.66 ± 1.33	7.71 ± 1.57	10.49 ± 0.77	9.52 ± 1.40
Me17:0 isomer	2.35 ± 0.57	2.26 ± 0.11	2.57 ± 0.29	2.04 ± 0.25
i17:0	2.78 ± 0.08	3.56 ± 0.49	3.05 ± 0.21	3.01 ± 0.16
ai17:0	2.75 ± 0.19	3.05 ± 0.33	3.15 ± 0.11	2.97 ± 0.15
17:0 c	3.08 ± 0.19	3.05 ± 0.27	2.83 ± 0.15	3.33 ± 0.26
17:1 isomer	1.25 ± 0.28	2.26 ± 0.29	2.00 ± 0.19	1.74 ± 0.19
17:0	1.30 ± 0.18	1.81 ± 0.17	1.56 ± 0.15	1.48 ± 0.11
17:0 isomer	1.61 ± 0.20	2.29 ± 0.13	2.29 ± 0.12	1.93 ± 0.22
18:0 isomer	1.58 ± 0.27	2.05 ± 0.28	1.73 ± 0.08	1.71 ± 0.10
18:2ω6 c	4.44 ± 0.44	3.56 ± 0.24	2.30 ± 0.70	3.42 ± 0.27
18:1ω9 c	9.94 ± 0.94	7.06 ± 0.42	6.36 ± 0.70	7.95 ± 0.56
18:1ω9 t	13.68 ± 0.50	8.97 ± 0.57	8.62 ± 1.14	10.72 ± 0.39
18:1ω7 t	1.56 ± 0.15	2.97 ± 0.82	2.46 ± 0.41	2.30 ± 0.14
18:1 isomer	2.10 ± 0.22	3.63 ± 1.28	2.81 ± 0.30	2.66 ± 0.29
18:0	5.18 ± 0.47	6.54 ± 1.53	6.71 ± 0.41	5.78 ± 0.59
19:2	2.39 ± 0.29	3.29 ± 0.16	3.32 ± 0.21	3.16 ± 0.24
19:0 c	7.10 ± 0.79	8.68 ± 0.89	9.90 ± 1.51	8.49 ± 0.37
19:0	1.35 ± 0.29	2.63 ± 0.38	2.63 ± 0.33	1.79 ± 0.22
20:0	2.78 ± 0.49	4.29 ± 1.01	3.12 ± 0.31	4.27 ± 1.44

Table AIV.7: Mean of each PLFA biomarker, recorded as mol % from each depth band on fairways at John O'Gaunt Golf Club, John O'Gaunt course. Surveyed in March 2006, n=5 per surface.

	Depth			
Biomarker	0 – 75 mm	76 – 150 mm	151 – 225 mm	0 – 225 mm
14:0	1.09 ± 0.04	1.07 ± 0.10	1.17 ± 0.13	0.96 ± 0.09
14:1 isomer a	1.28 ± 0.14	1.46 ± 0.17	1.77 ± 0.24	1.10 ± 0.13
14:1 isomer b	0.83 ± 0.10	1.15 ± 0.14	1.38 ± 0.22	0.83 ± 0.10
i15:0	4.89 ± 0.67	3.30 ± 0.52	3.05 ± 0.35	3.46 ± 0.38
ai15:0	3.71 ± 0.54	3.06 ± 0.43	3.26 ± 0.34	2.98 ± 0.31
15:1	1.11 ± 0.24	1.75 ± 0.22	1.80 ± 0.22	1.39 ± 0.27
15:0	1.42 ± 0.08	1.33 ± 0.10	1.31 ± 0.10	1.29 ± 0.04
16:1 isomer	0.96 ± 0.24	1.39 ± 0.13	1.69 ± 0.25	0.96 ± 0.08
i16:0	4.02 ± 0.31	3.98 ± 0.61	4.41 ± 0.32	3.54 ± 0.21
16:1ω7 c	5.53 ± 0.16	4.74 ± 0.55	4.97 ± 0.78	4.58 ± 0.18
16:1ω7 t	1.12 ± 0.31	1.99 ± 0.43	2.28 ± 0.38	0.93 ± 0.08
16:0	12.00 ± 1.38	7.04 ± 1.40	7.23 ± 1.13	10.12 ± 1.16
Me17:0 isomer	2.23 ± 0.48	2.91 ± 0.30	2.72 ± 0.51	2.45 ± 0.66
i17:0	2.58 ± 0.24	3.02 ± 0.09	2.93 ± 0.15	2.55 ± 0.09
ai17:0	2.64 ± 0.29	2.72 ± 0.30	3.25 ± 0.10	2.54 ± 0.14
17:0 c	2.93 ± 0.35	2.62 ± 0.18	2.84 ± 0.18	2.58 ± 0.14
17:1 isomer	1.60 ± 0.34	1.98 ± 0.15	2.16 ± 0.26	1.54 ± 0.15
17:0	1.21 ± 0.24	1.46 ± 0.09	1.49 ± 0.16	1.15 ± 0.05
17:0 isomer	1.73 ± 0.20	2.26 ± 0.25	2.46 ± 0.37	2.16 ± 0.21
18:0 isomer	2.18 ± 0.18	2.04 ± 0.06	2.64 ± 0.60	2.07 ± 0.09
18:2ω6 c	4.22 ± 0.77	2.56 ± 0.42	2.68 ± 0.36	3.43 ± 0.32
18:1ω9 c	9.39 ± 0.70	6.12 ± 0.55	5.93 ± 0.61	9.28 ± 1.12
18:1ω9 t	8.28 ± 0.15	6.60 ± 0.39	6.80 ± 0.54	8.40 ± 0.57
18:1ω7 t	1.74 ± 0.24	2.53 ± 0.28	2.59 ± 0.37	2.33 ± 0.22
18:1 isomer	1.62 ± 0.30	2.70 ± 0.43	2.63 ± 0.28	2.29 ± 0.29
18:0	4.80 ± 1.35	7.97 ± 2.86	4.79 ± 0.63	4.83 ± 0.59
19:2	2.46 ± 0.36	3.11 ± 0.29	3.32 ± 0.19	3.03 ± 0.23
19:0 c	9.01 ± 2.48	11.90 ± 1.45	10.70 ± 0.66	12.68 ± 0.99
19:0	1.44 ± 0.48	2.56 ± 0.40	2.98 ± 0.38	1.70 ± 0.21
20:0	1.96 ± 0.38	2.70 ± 0.22	2.76 ± 0.26	2.86 ± 0.78

Table AIV.8: Mean of each PLFA biomarker, recorded as mol % from each depth band on greens at John O'Gaunt Golf Club, John O'Gaunt course. Surveyed in March 2006, n=5 per surface.

	Depth			
Biomarker	0 – 75 mm	76 – 150 mm	151 – 225 mm	0 – 225 mm
14:0	1.09 ± 0.15	1.29 ± 0.15	1.31 ± 0.18	1.31 ± 0.19
14:1 isomer a	1.31 ± 0.06	1.86 ± 0.06	1.76 ± 0.28	1.83 ± 0.32
14:1 isomer b	0.90 ± 0.5	1.16 ± 0.08	1.26 ± 0.22	1.28 ± 0.26
i15:0	3.85 ± 0.54	2.96 ± 0.48	2.79 ± 0.33	2.75 ± 0.22
ai15:0	3.04 ± 0.38	2.98 ± 0.41	2.84 ± 0.36	2.82 ± 0.26
15:1	1.19 ± 0.09	1.48 ± 0.14	1.75 ± 0.30	1.60 ± 0.29
15:0	1.16 ± 0.08	1.06 ± 0.30	1.27 ± 0.12	0.95 ± 0.27
16:1 isomer	1.06 ± 0.09	1.39 ± 0.15	1.44 ± 0.17	1.45 ± 0.28
i16:0	2.64 ± 0.19	2.43 ± 0.18	2.64 ± 0.40	3.07 ± 0.51
16:1ω7 c	5.38 ± 0.41	5.16 ± 0.26	4.20 ± 0.33	4.50 ± 0.20
16:1ω7 t	1.30 ± 0.08	1.49 ± 0.07	2.06 ± 0.38	1.96 ± 0.60
16:0	14.56 ± 0.89	11.23 ± 1.32	8.01 ± 0.98	9.77 ± 1.33
Me17:0 isomer	2.23 ± 0.35	1.94 ± 0.15	2.36 ± 0.27	3.28 ± 0.86
i17:0	3.03 ± 0.09	3.49 ± 0.21	2.88 ± 0.29	3.25 ± 0.08
ai17:0	2.95 ± 0.6	3.29 ± 0.11	3.01 ± 0.24	3.52 ± 0.09
17:0 c	3.65 ± 0.16	3.94 ± 0.14	3.15 ± 0.18	3.42 ± 0.31
17:1 isomer	1.33 ± 0.04	1.98 ± 0.18	2.45 ± 0.30	2.33 ± 0.36
17:0	1.54 ± 0.05	1.78 ± 0.16	1.61 ± 0.14	1.98 ± 0.14
17:0 isomer	1.49 ± 0.030	2.32 ± 0.13	2.32 ± 0.13	2.12 ± 0.10
18:0 isomer	1.40 ± 0.06	1.95 ± 0.16	1.99 ± 0.09	1.92 ± 0.11
18:2ω6 c	3.99 ± 0.37	2.66 ± 0.10	3.04 ± 0.35	2.93 ± 0.27
18:1ω9 c	10.57 ± 0.80	6.67 ± 0.50	7.41 ± 1.95	6.72 ± 1.07
18:1ω9 t	8.75 ± 0.29	8.40 ± 0.45	6.58 ± 0.82	7.14 ± 0.99
18:1ω7 t	2.56 ± 0.20	2.90 ± 0.13	2.63 ± 0.53	2.53 ± 0.23
18:1 isomer	1.60 ± 0.11	2.13 ± 0.19	3.22 ± 0.74	2.30 ± 0.27
18:0	4.88 ± 0.35	5.57 ± 0.46	8.56 ± 2.16	5.54 ± 0.49
19:2	1.98 ± 0.04	2.96 ± 0.23	2.85 ± 0.33	2.75 ± 0.04
19:0 c	7.13 ± 0.38	8.04 ± 0.71	8.43 ± 1.67	7.81 ± 0.75
19:0	1.40 ± 0.05	2.21 ± 0.37	2.80 ± 0.54	2.99 ± 0.51
20:0	2.01 ± 0.06	3.26 ± 0.35	3.40 ± 0.39	4.16 ± 1.06

Table AIV.9: Mean of each PLFA biomarker, recorded as mol % from each depth band on areas of the rough at John O'Gaunt Golf Club, John O'Gaunt course. Surveyed in March 2006, n=5 per surface.

	Depth			
Biomarker	0 – 75 mm	76 – 150 mm	151 – 225 mm	0 – 225 mm
14:0	0.89 ± 0.06	1.06 ± 0.13	1.35 ± 0.12	1.15 ± 0.16
14:1 isomer a	0.95 ± 0.07	1.24 ± 0.19	1.79 ± 0.26	1.69 ± 0.31
14:1 isomer b	0.75 ± 0.08	1.11 ± 0.24	1.30 ± 0.15	1.23 ± 0.21
i15:0	3.70 ± 0.53	3.06 ± 0.48	2.96 ± 0.50	3.29 ± 0.39
ai15:0	2.72 ± 0.12	2.78 ± 0.21	3.01 ± 0.30	2.96 ± 0.38
15:1	1.08 ± 0.14	1.58 ± 0.23	1.95 ± 0.17	1.51 ± 0.19
15:0	1.37 ± 0.11	1.48 ± 0.11	1.64 ± 0.08	1.39 ± 0.12
16:1 isomer	0.94 ± 0.10	1.22 ± 0.19	1.58 ± 0.18	1.31 ± 0.18
i16:0	3.73 ± 0.50	3.44 ± 0.82	3.46 ± 0.77	3.75 ± 0.74
16:1ω7 c	4.92 ± 0.21	4.07 ± 0.15	4.16 ± 0.40	4.00 ± 0.40
16:1ω7 t	1.08 ± 0.20	1.19 ± 0.13	1.76 ± 0.24	1.73 ± 0.59
16:0	9.94 ± 0.59	7.77 ± 0.76	7.55 ± 1.47	8.99 ± 0.83
Me17:0 isomer	4.00 ± 0.88	4.45 ± 0.75	3.47 ± 0.70	4.20 ± 0.75
i17:0	2.39 ± 0.13	2.78 ± 0.05	2.92 ± 0.25	2.64 ± 0.21
ai17:0	2.47 ± 0.09	2.90 ± 0.11	3.30 ± 0.19	3.08 ± 0.18
17:0 c	2.78 ± 0.18	2.83 ± 0.19	2.90 ± 0.09	2.81 ± 0.22
17:1 isomer	1.61 ± 0.13	1.98 ± 0.18	2.32 ± 0.15	1.97 ± 0.16
17:0	1.25 ± 0.10	1.40 ± 0.19	1.65 ± 0.14	1.49 ± 0.18
17:0 isomer	2.08 ± 0.15	2.66 ± 0.28	2.84 ± 0.34	2.49 ± 0.38
18:0 isomer	2.29 ± 0.15	2.56 ± 0.15	2.69 ± 0.24	2.59 ± 0.30
18:2ω6 c	3.44 ± 0.39	2.63 ± 0.10	2.96 ± 0.27	2.90 ± 0.25
18:1ω9 c	10.36 ± 0.77	7.64 ± 0.54	5.46 ± 0.23	7.22 ± 0.68
18:1ω9 t	8.57 ± 0.56	7.24 ± 0.57	5.62 ± 0.37	6.91 ± 0.69
18:1ω7 t	1.71 ± 0.18	2.69 ± 0.39	2.85 ± 0.37	2.07 ± 0.08
18:1 isomer	1.96 ± 0.24	2.71 ± 0.56	2.40 ± 0.34	2.17 ± 0.29
18:0	4.14 ± 0.29	3.71 ± 0.53	6.03 ± 0.67	4.57 ± 0.23
19:2	2.84 ± 0.28	3.24 ± 0.28	3.48 ± 0.36	2.93 ± 0.13
19:0 c	11.79 ± 0.87	13.33 ± 0.86	10.07 ± 1.29	11.57 ± 1.75
19:0	1.55 ± 0.19	2.26 ± 0.43	2.92 ± 0.44	2.30 ± 0.39
20:0	2.68 ± 0.34	2.98 ± 0.36	3.60 ± 0.31	3.09 ± 0.33

Appendix V: *Lumbricus terrestris* (L) behavioural studies

The following observations of earthworm casts frequencies were made in microcosm investigations described in Chapters 8 and 9, conducted at Cranfield University at Silsoe experimental greenhouses.

Profile construction

Table AV.1: Frequency table of earthworm casts recorded on microcosms investigating the effect of different construction profiles. Observed vs. expected frequencies proportion of linear covariance: $r^2 = 0.994$.

Number of casts observed per microcosm	Count	Cumulative Count	Cumulative Percent
0	269	269	74.7
1	58	327	90.8
2	21	348	96.7
3	7	355	98.6
4	5	360	100.0

Physical exclusion

Table AV.2: Frequency table of earthworm casts recorded on microcosms investigating the effect of physical exclusion barriers. Observed vs. expected frequencies proportion of linear covariance: $r^2 = 0.999$.

Number of casts observed per microcosm	Count	Cumulative Count	Cumulative Percent
0	484	484	67.2
1	181	665	92.4
2	45	710	98.6
3	9	719	99.9
4	1	720	100.0

The vertical distribution of the earthworms that remained in the experiment was analysed using a GLM with a construction x barrier depth interaction. No significant differences in the distribution of *L. terrestris* was recorded (Table 9.3), no trend was evident in the residuals (Figure V.1).

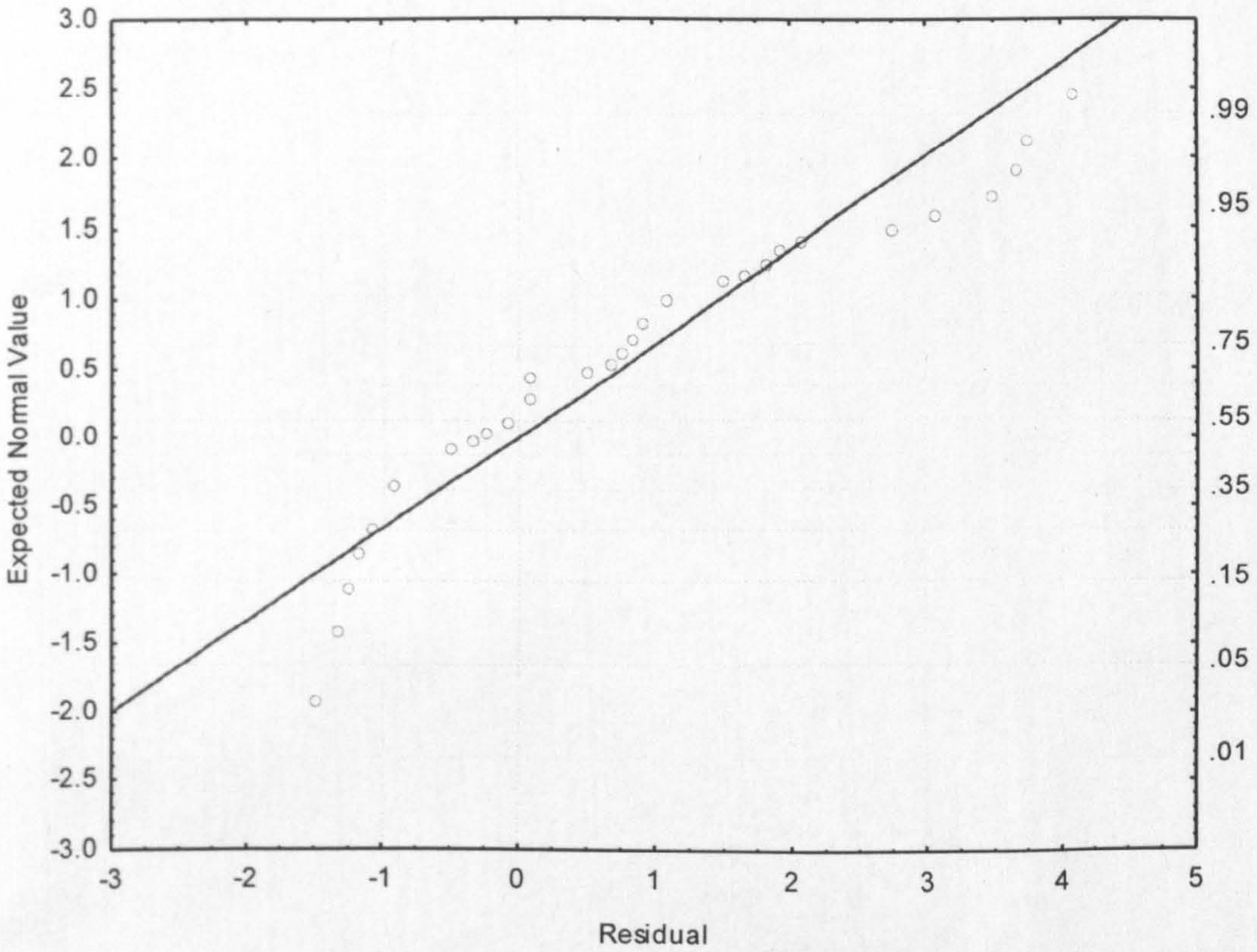

Figure AV.1: P-plot of residuals from the GLM analysis carried out in Chapter 9.3, investigating the vertical distribution of earthworms in microcosms with physical exclusion barriers.

Soil microbial control

Table AV.3: Frequency table of earthworm casts recorded on microcosms investigating the soil microbial control methods. Observed vs. expected frequencies proportion of linear covariance: $r^2 = 0.999$.

Number of casts observed per microcosm	Count	Cumulative Count	Cumulative Percent
0	297	297	87.4
1	37	334	98.2
2	6	340	100.0