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The effects of landfill leachate irrigation on the physio-chemical properties of Oxford clay and physiology of coppiced *Salix viminalis*

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Abstract

The irrigation of *Salix* species with landfill leachate, when grown on landfill sites, is a financially attractive solution to leachate treatment and landfill site remediation. Detailed experiments were carried out in the summers of 2001 and 2002 in which *Salix viminalis*, growing in lysimeters, received >100mm per week irrigation with landfill leachate of electrical conductivity 12-13 $DS.m^{-1}$. The aim was to investigate the physiological effects of heavy leachate irrigation and determine a simple mechanism to relate these effects to salt loading. Incorporation of a temporal factor into EC ($S.m^{-1}.day$) resulted in reliable correlations between EC days and salt addition ($R^2 = 0.96$), soil matric potential ($R^2 = 0.89$), foliar gas exchange ($R^2 > 0.72$) and leaf area ($R^2 = 0.78$). Persistent leachate treatment resulted in extensive defoliation but a 236% enhancement of dry weight relative to water only irrigation. Foliar gas exchange of leachate irrigated plants was suppressed more rapidly than in water deprived treatment, although the response to leachate and droughting was broadly similar .

Destructive analysis of lysimeters from each treatment revealed diverse physio-chemical soil conditions. The leachate irrigated treatment showed elevated concentrations of soil nitrate (336 fold), ammonium (30 fold) and chloride (10 fold) relative to the water only irrigation treatment. All irrigation treatments had properties which were indicative of a soil which is inhibitory to liquid and gas movement as shown by oxygen diffusion rate (< 20 $g.cm^{-2}.s^{-1}$) and redox potential (> 273 mV). After four weeks irrigation with bore water or under natural precipitation a reduction of soil salts to the original levels was recorded.

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% = Percentage
a = Total surface area of the platinum electrode (cm^2)
A = Calculated carboxylation
ABA = Absissic acid
ATM = Atmospheric pressure
ATP = Adenosine triphosphate
b = Bar
C = Celsius
Ca = cuvette CO_2 concentration
CAM = Crassulacean acid metabolism
Ci = Sub stomatal CO_2 concentration
DC = Direct current
DW = Dry weight
E = Transpiration
EC = Electrical conductivity
$ECp_{(comp)}$ = desired compensated EC
$ECp_{(uncomp)}$ = calculated EC uncompensated for temperature
Ed = Edition
Eh = Redox potential
F = Faraday’s constant
Fm = Fluorescence (maximum)
Fv/Fm = Variable fluorescence/maximum fluorescence
fx = Oxygen diffusion rate to the platinum electrode (cm^{-1}.min^{-1})
g = Gram
Gs = Stomatal conductance
hrs = Hours
i = Measured current (amperes)
IRGA = Infra red gas analyser
Km = Enzyme velocity
l = litre
L = Length
Ln = Natural logarithm
LSD = Least significant difference
m = Metre
M = Molar
mol = Molecule
n = Number of electrons needed for the reduction of 1 molecule of oxygen (n = 4)
NADP = Nicotinamide Adenine Dinucleotide Phosphate
No. = Number
ODR = Oxygen diffusion rate
p = probability
Pa = Pascals
PAR = Photosynthetically active radiation
pH = Positive hydrogen’s
ppm = Parts per million
R^2 = Correlation coefficient

RuBISCO = Ribulose biphosphate carboxylase/oxygenase
S = Seimen
s = Second
SBA = Stem basal area
SE = Standard error (of mean)
sp. = Species
t = temperature
TM = Trademark
V = (Reading from Auto analyser/(dilution) – Blank
V = Volts
W = Width
Δ = Change (in)
Σ_b = Electrical permittivity of the bulk soil
Σ_p = Electrical permittivity of the soil pore water
$\Sigma_{\sigma b=0}$ = Offset soil parameter
σ_b = Electrical conductivity of the bulk soil
σ_p = Electrical conductivity of the pore water

1 Introduction

Species from the *Salicaceae* family have been useful to mankind for centuries (Stott, 1992). More recently the ability of *Salix sp.* to sequester large amounts of nutrients has been exploited by land managers to purify waters and soils (Perttu and Kowalik, 1997 and Elowson, 1999), to revegetate areas contaminated with radionuclide deposits (Goor *et al*,. 2001) to phytoremediate aquifiers contaminated with ethanol blended gasoline (Corseuil and Moreno, 2001) and as a landfill based leachate attenuation system (Ettala, 1988).

Biomass crops are particularly appropriate for phytoremediation purposes because of their high consumptive water use. There is an increased likelihood of a larger proportion of the fugitive chemicals in a contaminated soil being included into the transpiration stream, and therefore incorporated into the plant tissues. Furthermore, biomass crops would be repeatedly harvested for combustion or digestion and thus the soil contaminants will be removed. Of particular importance to total plant evpotranspiration is active leaf area. This is comprised of two components: the physical leaf area and individual leaf gas exchange. Combined these control the total plant evapotranspiration and the effectiveness of a plant based evaporative filter. In addition to factors associated with water vaporisation, photosynthetic gas exchange is of particular importance as it is directly related to the accumulation of biomass and plant condition.

Wastewater or landfill leachate irrigation invariably involves a large amount of salts being introduced into a system. This has repercussions not only on the physical and chemical properties of a soil but also on organisms above and below ground. To maximise the effectiveness of an evaporative filter an understanding of the implications of wastewater irrigation on soil and plant condition is of critical importance. A number of papers have highlighted the potential for a landfill based leachate attenuation system and confirmed the need for appropriate management of the ecosystem (Shrive *et al.,* 1990, Chan *et al.,* 1998 and Cureton *et al.,* 1991). In particular, salt addition has been identified potentially the main constraint to a landfill leachate attenuation system (Stephens *et al.,* 2000). An upper limit of leachate irrigation has not been determined, this would be necessary to avoid excessive leachate application.

Given the uncertainties surrounding heavy leachate irrigation this study investigated the effects of landfill leachate irrigation on soil physio-chemical properties and plant physiology and therefore aimed to inform practitioners on the scheduling of leachate delivery and determine the upper threshold of *Salix* tolerance to landfill leachate irrigation.

2 General experimental design

2.1 Introduction

The following chapter outlines some of the fundamental experimental designs common to all the subsequent chapters.

Figure 2.1: The experimental plot showing the lysimeters supporting one year old *Salix viminalis* (Cranfield University, Silsoe, Bedfordshire) in May 2001.

2.2 Lysimeter composition

Lysimeters were used in the experiment primarily because they facilitated relatively accurate leachate delivery and effluent collection, although they were initially established for a separate study. The lysimeters (see Figure 2.1) were filled with 180 kg of recently excavated Oxford clay obtained from the Brogborough (Bedfordshire, UK) pit face. Oxford clay was used because of future plans by Shanks^{TM} to cap the Brogborough landfill site with this substrate.

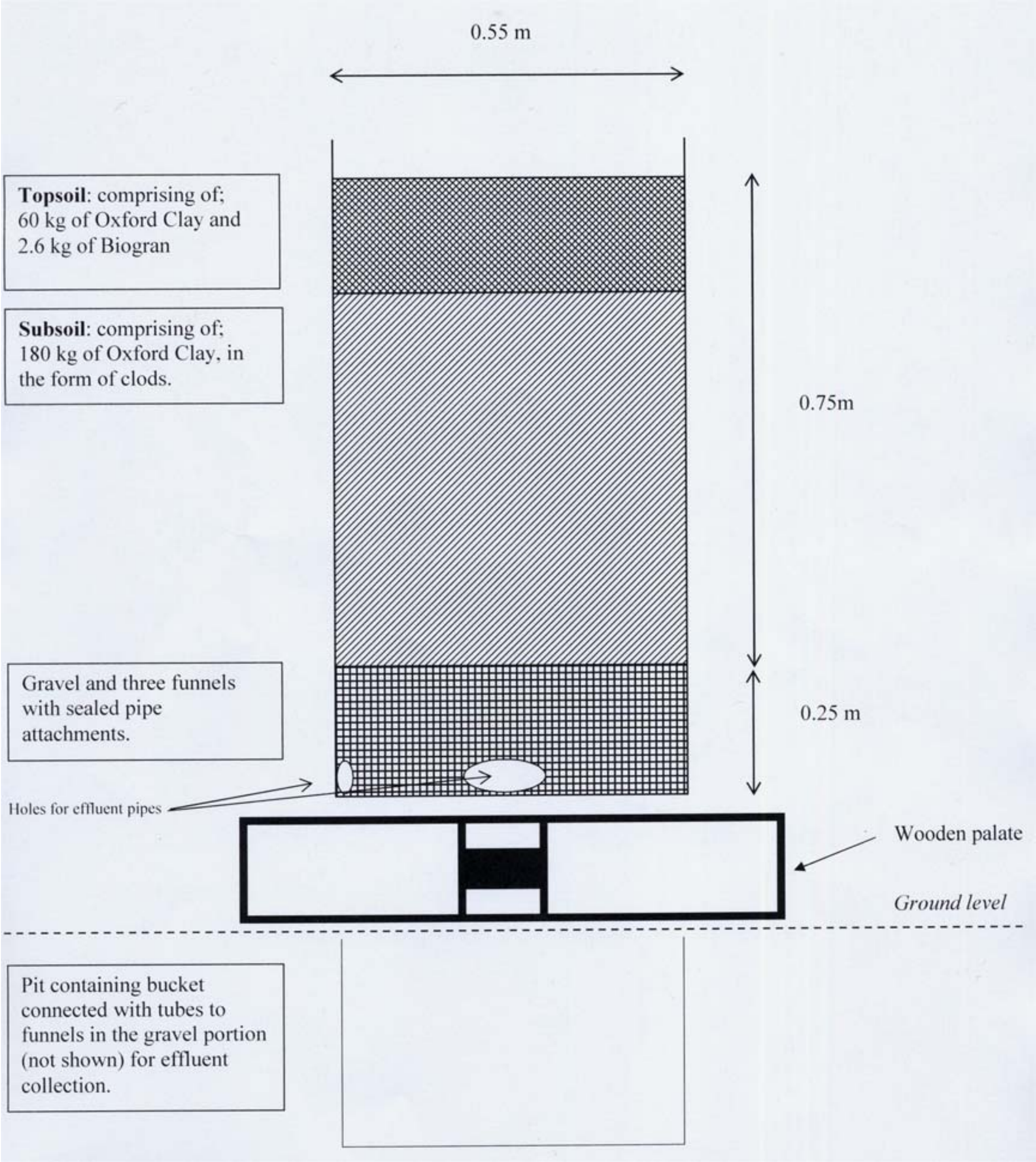

Figure 2.2: Specifications of a typical lysimeter used.

The soil was packed with fist sized clods of Oxford clay and an additional 60 kg of clay mixed with 2.6 kg Biogran^{TM} (dried sewerage sludge) made up the upper horizon in an arrangement indicated by Figure 2.2. After saturation the lysimeters settled to an estimated bulk density of approximately 1450 kg.m^{-3}.

The gravel filled base with integrated funnels and sub surface collection pit was of special importance as this facilitated free flowing conditions needed for effluent collection which was a component of an associated study.

2.3 Tree layout

A randomised block design was applied to the treatments which received a variety of leachate irrigation regimes. The experimental design was constructed and implemented prior to the start of the experiments described in this thesis.

2.4 Leachate chemical composition

Leachate is inherently variable in its composition, previous published articles have analysed the leachate used for a number of properties. Due to the origin of the leachate used and magnitude of leachate irrigation in the experiment, only nitrogen and chloride components were analysed at regular intervals.

Table 2.1: Average leachate compositions, taken from a) Harrington and Maris, (1986), b) WRC (2002), c) Cureton *et al.*, (1991) d) Shrive *et al.*, (1990) all values are presented as mg.l^{-1} unless stated and ND = not determined.

Constituent	*Aged wastes*^{a}	*Hatfield leachate*^{b}	*Guelph leachate*^{c}	*Muskoka leachate*^{d}	*Dogsthorpe leachate*
pH	7.5	7.3	6.7	5.4	7.6
COD (mg O.l^{-1})	700	651	14300	ND	1991
BOD (mg O.l^{-1})	70	44.2	ND	ND	103
Ammonical N	260	85.7	ND	103	36.9
Oxidised N	7.5	0.6	ND	0	2.2
Chloride	1400	889	1.2	98	1761
Sodium	880	549	0.9	41	1336
Magnesium	130	107	0.5	32	118
Potassium	340	123	1.3	114	702
Calcium	200	372	0.9	ND	143
Chromium	0.07	0.04	0.001	ND	ND
Manganese	1.7	ND	0.4	6.8	118
Iron	10	ND	0.06	37.5	ND
Zinc	0.2	0.03	0.002	2.7	ND

Bore water was also analysed for chloride (average 63.2 mg.l^{-1}) nitrate (average 2.9 mg.l^{-1}) and ammonium (average 18.6 mg.l^{-1}). The average EC of the leachate was 13.4 DS.m^{-1}.

2.4.1 Irrigation

Irrigation was achieved via calibrated and timed delivery using an irrigation system which utilised a constant head. In the 2001 experimental season irrigation commenced on 29th May and lasted for 16 weeks. In 2002 irrigation commenced on 8th April and lasted for 20 weeks. The irrigation delivery was calibrated every time the leachate tanks were changed after the irrigation network was washed out. The irrigation amount was adopted in order to keep the soil saturated and was determined by calculation of the expected evapo-transpiration (P. Martin pers. com, 2001). Subsequently, the irrigation delivery was modified at different times in the season.

Two treatments were employed in the 2001 the conrol which received bore water and one which received landfill leachate (Dogsthorpe, Cambridgeshire). In 2002 two more treatments were added these treatments underwent cyclical leachate:water irrigation; L1 which was irrigated with bore water instead of leachate for five weeks in the 2002 season and consequently received 87% of the leachate received by the persistant leachate treatment, and cyclical leachate:water which was irrigated with bore water instead of leachate for ten weeks in the 2002 season and consequently received 72% of the leachate received by the persistant leachate treatment. Each treatment received the same depth (2500 mm) of irrigation. The cumulative depth of irrigation over the 2002 experimental period was 10 times more than Shrive *et al.* (1990) and approximately 4 times more than Cureton *et al.* (1991) and Dimitriou and Aronsson (2004).

2.5 Plant material

Salix viminalis is noted for its relative resistance to rust attacks[1] relative to other *Salix* sp. and its ability to grow well on nutrient poor and high pH soils. Furthermore *Salix viminalis* "Jorr" was the most salt tolerant species available at the commencement of the experimental period.

On the 21st April 1999 cuttings of *Salix viminalis* "Jorr" where planted in the lysimeters. Each lysimeter received one cutting approximately 0.25 m long which was planted centrally to a depth of approx. 0.2 m. They were cut back for the first and only time in the course of the 3 year experiment on the 6th December 1999. On this date each clone was cut to 0.05 m from the surface in order to simulate the action of a mechanical harvester (P. Thorn pers. com. 2001).

2.6 Experimental management

During the experiment there were no plans to use pesticides or herbicides, however outbreaks of greenfly (*Macrosiphum rosae)* and black willow aphid (*Melanoxantherium salicis)* were frequently found on the willows. Fearing a possible interference with the performance of the trees, dilute detergent and removal by hand was used to prevent an epidemic. Frequently weeds would germinate in the lysimeters; these were dealt with via hand pulling and returning as much soil as possible back to the lysimeter. Furthermore leaves would deposit in the lysimeters, these also were removed by hand.

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[1] Outbreaks of *Melanspora* rust have devastated hectares of SRC land in Long Ashton research institute (Hall *et al.,* 1996).

2.7 Treeless control

Prior to the commencement of the 2001 irrigation period, a treeless control experiment spanning five weeks was completed. The aim of this study was to investigate the effects of landfill leachate irrigation on the soil physio-chemcial properties without the presence of *Salix viminalis*. This would inform about the role of the plant in moderating performance of the soil system.

Scaled down lysimeters (1:12) were packed with Oxford clay to a comparable bulk density and composition to the field lysimeters. Leachate and water irrigation was delivered via a drip irrigation system at a scaled down depth to the field scale lysimeter but at the same frequency. Effluent was collected daily from each lysimeter and regular Electrical Conductivity measurements were taken in the 0.1m horizon throughout the experiment. After five weeks of irrigation the lysimeters were split open and soil chemical measurements were obtained in a split plot manner from the core of each lysimeter.

2.8 Data Management

The data was statistically analysed using analysis of variance (Genstat 5th Ed. Adept scientific, Rothamsted research), or regression analysis (Statistica, Statsoft)

3 Soil conditions throughout the irrigation season

3.1 Introduction

Irrigation of crops with landfill leachate will result in substantial loading of salts onto the plant substrate which results in an increase in osmotic potential of the substrate. Therefore, for sustainable management of a landfill leachate attenuation system a suitable method for the quantification of salt deposition needs to be determined.

3.2 Literature Review of soil conditions under leachate irrigation

Electrical conductivity (EC) is a measure of the ease of a current to pass through a substance. EC has been used to map seawater incursion (Nobes, 1996), groundwater contamination (Drommerhauser *et al.*, 1995) and nitrogen levels in soils low in salts and free carbonates (Zhang and Weinhold, 2002). Furthermore, EC has been used in studies of soil salinity and irrigant salinity (De Jong *et al.*, 1979, Rhodes and Corwen, 1981, Adams *et al.,* 1993 and Bassil and Kaffka, 2002). EC itself has no effect on plant welfare but it is related to factors which do i.e. salinity and nutrient status. Conductivity may be measured in a number of ways: frequency domain methods, georadar or ground penetrating radar (Darayan *et al.,* 1998) resistivity probes (Yoon and Park, 2001), capacitance and theta probes (Robinson *et al.,* 1999). Commonly measurement of EC (typically expressed as $S.m^{-1}$) centres on the transference of an applied voltage across a conductivity bridge and subsequent measurement of the current. This technique can be applied to the measurement of EC in soil in two laboratory based ways:

- Saturated paste method (US Salinity Lab staff 1954, MAFF, 1986)
- Soil:water extract method (Dahnke and Whitney, 1988, Dellavalle, 1992).

Both methods require specialist equipment and are relatively time consuming. Of the two, the saturated paste method is more representative of the soil solution under field conditions. However, it is more time consuming than the soil:water extract method and more difficult to reproduce across a wide range of soil types. Therefore there was a niche in the market for an *in situ* soil EC probe.

The Delta-t (Cambridge, UK) ECK-1 Sigmaprobe is one such portable EC probe, it is capable of taking rapid *in situ* measurements of soil pore water conductivity without prior specialist knowledge. The Sigmaprobe ECK-1 does this by measuring the permittivity, electrical conductivity of the bulk soil (at a frequency 30MHz) and temperature of the soil. Due to the linear relationship between temperature and permittivity of the soil water, calculation of the water permittivity can take place. The permittivity of water, coupled with the measured bulk soil permittivity and conductivity can be used to calculate pore water conductivity using Equation 1:

$$\sigma_p = \Sigma_p \, \sigma_b / (\Sigma_b - \Sigma_{\sigma b=0})$$

Equation 3.1: The equation used to derive ECp from the measured parameters of the ECK-1 sigmaprobe, where Σb Electrical permittivity of the bulk soil, Σp = Electrical permittivity of the soil pore water, $\Sigma_{\sigma} b_{=0}$ = Offset soil parameter, σb =Electrical conductivity of the bulk soil, σp = Electrical conductivity of the pore water (Delta-t, 2001)

The key influences on EC in soil water solution are the concentration and velocity of electrolytes, which is particularly influenced by soil bulk conductance, moisture content, ion content, soil temperature and compaction (Yoon and Park, 2001 and Nobes, 1996). EC is used increasingly for agronomic purposes; Table 3.1 demonstrates a classification structure.

Table 3.1: The classification of soil salinity taken from the Delta-t (2001)

Description	*Measured EC (DS.m^{-1})*
Non saline	0-2
Slightly Saline	2-4
Moderately Saline	4-8
Strongly saline	8-16
Extremely saline	>16

The increase in use of EC can be attributed to more responsible nutrient management, arable extension to saline soils and irrigation of crops with saline solutions in order to meet the shortfall in crop water demand in arid areas. De Jong *et al.* (1979) and Nelson and Ham (2000) have demonstrated that an increase in salinity has resulted in a decrease in consumptive water use and photosynthesis. Therefore, with such large volumes of chloride being applied and the likelihood of chloride being the main constraint to plant productivity of a landfill leachate recirculation system (Stephens *et al.,* 2000) it is intuitive to suggest that salinity be the principal soil parameter to be measured during the experimental period.

Irrigation with leachate not only imports large volumes of chloride salts but also a number of other important salts. One of the most important salts in a landfill leachate recycling system is ammonium. Guidelines e.g. MAFF (1984) published for the application of nitrogen are dependent on the previous years crop, the current crop, cost:benefit ratio and the timing of application. Nevertheless in 2000 the average total nitrogen application for Great Britain was 123 kg.ha^{-1}.

Temperature is influential in denitrification (Ilies and Mavinic, 2001) and physiological response (Boone *et al.,* 1998 and Ziska, 1998) furthermore it is of special importance to landfill based leachate attenuation system as subsurface decomposition may elevate soil temperature.

3.2.1 Water potential

Water plays a crucial role in the metabolism of a plant. Quantification of plant water status facilitates cross comparison between species and context of stress. One of the most important developments in plant science of the 20th century was the transition from describing plant water status in terms of osmosis (osmotic pressure) to water potential as this is a physically defined description of plant water status. Plant water potential is influenced by a number of environmental factors and is comprised of three intrinsic components as described by Equation 3.2:

$$\Psi w = \Psi s + \Psi p + \Psi g$$

Equation 3.2: The equation used to describe plant tissue water potential; where: Ψs = solute or osmotic potential, Ψp = pressure potential, Ψg = gravitational potential and Ψw = plant water potential.

The usefulness of water potential has been challenged because it allegedly is not well correlated with some plant processes (Kramer and Boyer, 1995). Some authors prefer to use relative water content as an indicator of plant water status, but water potential has the advantage of being physically defined and related to the factors/forces which control water movement. Typically water potential values (expressed in Pascals) are inversely proportional to the water status of the tissue sampled.

The measurement of water potential is typically achieved through the use of a pressure chamber; in which steady pressure is applied until the meniscus of the sap is apparent through the excised petiole e.g. Sellin (1996). Water potential varies diurnally; it is particularly influenced by soil water availability, incident radiation and time of day.

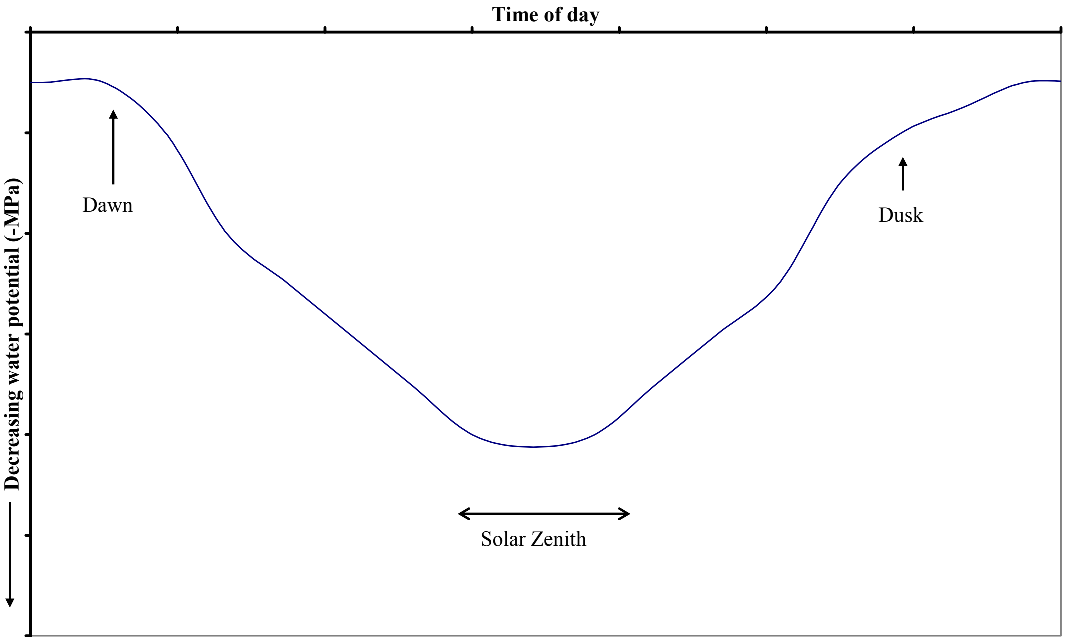

Figure 3.1: The typical diurnal change in foliar water potential.

When gaseous fluxes are at a minimum, relatively little force acts on the plant, therefore plant water status aligns with the available water. As incident radiation increases during the course of the day, the vapour pressure deficit (VPD) at a foliar level also increases, resulting in the evaporation of water and diminishment of plant water reserve, which results in an increase in water tension in the transpiration stream. Consequently, plant water potential will decrease until the solar zenith. Once the solar zenith has passed, a reduction in foliar VPD relieves some of the tension resulting from the evaporation of water in the transpiration stream and consequently the water potential increases see Figure 3.1. Measurement of water potential whilst there are no gas fluxes is thought to be correlated with the soil water status (Boyer 1995); in effect one is using the plant like a tensionmeter.

3.3 Objectives

The following objectives are to be fulfilled by the outlined experiments;

- To compare the EC of Oxford clay with the EC of other soil.
- To compare methods for measuring EC.
- To measure EC, temperature and permittivity at regular intervals during the irrigation period.
- To develop a comparable measure of the cumulative salt addition.

3.4 Method

3.4.1 EC protocol

Unless otherwise stated the EC was measured using a Delta-t ECK-1 Sigmaprobe at a depth of 0.1 m. No temperature compensation was applied at the point of measurement but rather temperature compensation was applied retrospectively to 20^{o}C using

Equation 3.3.

$$ECp_{(comp)} = ECp_{(uncomp)} \text{ x } (1 + 0.02 \text{ x } (20 - t))$$

Equation 3.3: To compensate the measured EC to 20^{o}C; where ECp(comp) = is the temperature compensated pore water EC , ECp(uncomp) = the uncompensated EC and t = measured temperature (E. Potter, Pers. com).

3.4.2 Sigmaprobe with standard method

Oven dried Oxford clay soil which was ground using a centrifugal grinder (Glencreaston/Retsch, Ball mill S100, Middlesex, UK) to pass through a 1 mm sieve, 250 g portions were weighed into beakers to which solutions of various EC's were added. A stock solution of a synthetic leachate was prepared (see appendix 9.1 for composition), and diluted with de-ionised water until the EC was comparable to that of the Dogsthorpe leachate.

Table 3.2: Different solutions used to induce a range of EC's see appendix 9.1 for recipe.

Solution	*%Water*	*% synthetic leachate*
1	100	0
2	75	25
3	50	50
4	25	75
5	0	100

From the stock solution further dilutions provided solutions with a range of EC values.

The appropriate solution was added to the beaker of soil and mixed thoroughly with the dried clay until the surface of the mixture glistened although the mixture wouldn't run if the beaker was tipped. The pore water EC was measured using the ECK-1 in the clay mixture in a number of locations repeated serially five times. A Buchner funnel and pump were employed to extract the soil water out of the clay:solution mix which was filtered through a Whatman No. 4 filter paper. The EC of the extract was then measured using the ECK-1 (Sigmaprobe, Delta-T equipment, Cambridge, UK) and a laboratory EC meter (Camlab CM-11P, Camlab, Cambridge, UK). Each treatment was replicated four times.

3.4.3 EC of different soil types

Four different soil types were located on the Silsoe College farm using King (1969) and G. Lovelace (pers. com. 2002). Once in the locality of the desired soil type 10 locations were selected randomly by throwing a paper packet and the electrical conductivity of the single location was taken serially five times using the standard ECK-1 protocol. (See section 3.4.1)

3.4.4 EC over the irrigation season

At 2 or 3 day intervals, measurements of the electrical conductivity of soil pore water in the wet zone of the lysimeters were taken using the protocol outlined in section 3.4.1. Serial measurements were repeated five times for one location per barrel. Furthermore 15 serial measurements were taken of the soil two metres from the experimental plot and 10 serial measurements of the leachate every time a new batch was connected to the irrigation system.

3.4.5 EC days

A cumulative measure of the stress imposed by leachate addition to the lysimeters was required. EC days was calculated by the summation of interval average of the recorded treatment EC over an experimental period relative to simultaneous measurement of the water control treatment (see Equation 3.4). The baseline EC is the average EC for the water controls obtained at the same periodicity as the treatment EC's.

$$\text{EC days} = \Sigma\,(EC_T - EC_C) \times (t_1 - t_2)$$

Equation 3.4: For calculation of EC days (DS m^{-1}.day) where; EC_T = compensated pore water EC of the treatment soil (DS.m^{-1}) EC_C = compensated pore water EC of the control soil (DS.m^{-1}) and (t_1- t_2) = the time delay in between simultaneous measurement of treatment and control pore water EC (days).

3.4.6 Equilibrium water potential

Water potential measurements were taken using a Scholander type pressure bomb, in accordance with Sellin, (1999). The chamber was prepared by greasing the bayonet fitting and inserting a moist piece of filter paper into the base of the chamber. Leaves from the smaller branches in the lower zone were excised from the abaxial to adaxial using a knife and immediately wrapped in cling film (W Stephens, pers. com. 2001). Once clamped into the bayonet fitting a steady pressure of 0.1 MPa a minute was applied. A more rapid application of pressure would result in bubbling.

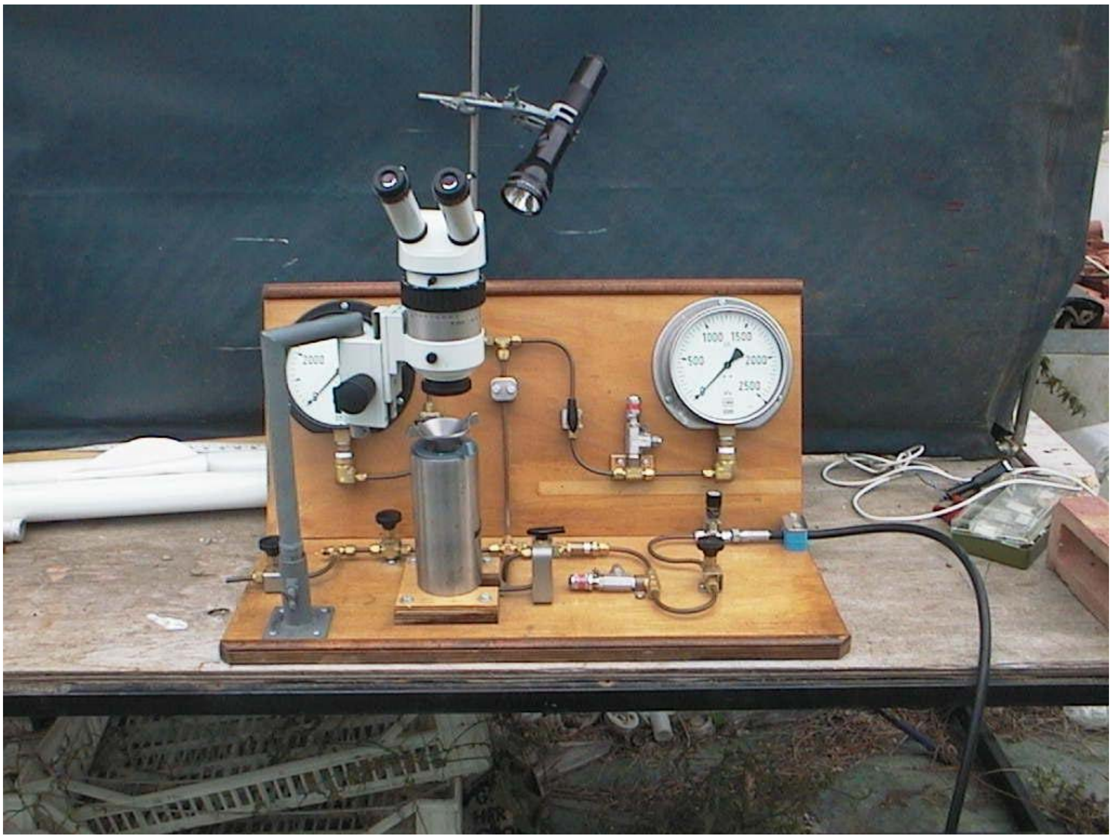

Figure 3.2: The Scholander type pressure bomb used for equilibrium water potential measurement, note the light source and dissecting microscope used for the identification of water meniscus.

At least 6 leaves from the lower section of the branches were sampled to obtain an equilibrium water potential, on a number of occasions in 2001 and 2002, (see Table 5.4).

3.5 Results

3.5.1 Sigmaprobe compared with standard method

Figure 3.3 demonstrates that there are no significant differences ($p = 0.196$) between the EC measured using the ECK-1 and the CM-11P in the extract resulting from the standard method.

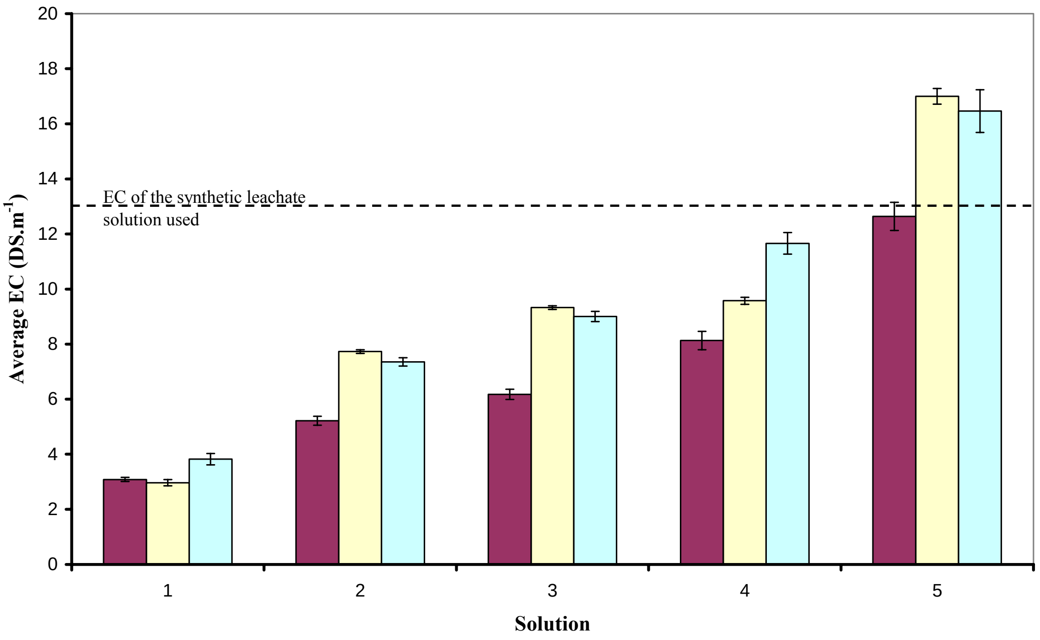

Figure 3.3: Average EC of saturated Oxford clay and resultant extracts using the ECK-1 and CMP-11, different solutions were introduced to induce a range of EC's. The EC of the extract measured using the ECK-1 meter is indicated by the yellow series, the extract EC measured using the CMP-11 is indicated by the blue series and the EC measured in the soil using the ECK-1 is the red series.

However a highly significant ($p < 0.001$) difference was apparent between the EC measured in the soil and the extract using the ECK-1 probe. Interestingly when the water solution was used to saturate the soil no significant differences occurred between the treatment EC means. The largest difference between the mediums for EC measurement occurred when the leachate solution 5 was used. Furthermore solution five resulted in an extract EC which had a higher average EC than the leachate, whereas the EC of the soil measured using the ECK-1 was just beneath the average EC of the solution.

3.5.2 Different soil types

Table 3.3 shows that there are significant differences between soil types. Furthermore, there are different EC's for the same sandy loam soil with different land use.

Table 3.3: Electrical conductivity of the soil pore water meassuresed using the ECK-1 for selected soils on the Silsoe College farm sampled on 09/10/01.

Soil type	*Land Use*	*Average EC (DS.m^{-1})*
Sandy loam	SRC Plot	2
Sandy loam	Arable	1.7
Gleyed brown earth	Arable	2
Brown earth	Arable	2.2
Clay	Agroforestry plot	2.7
Oxford clay soil	Clay tip	3.3

Finally, clay has a significantly higher ($p < 0.001$) electrical conductivity than the other tested soils.

3.5.3 EC of Oxford clay during leachate irrigation

Figure 3.3 illustrates that the persistent application of leachate results in a plateauing of EC well below the EC of the leachate applied.

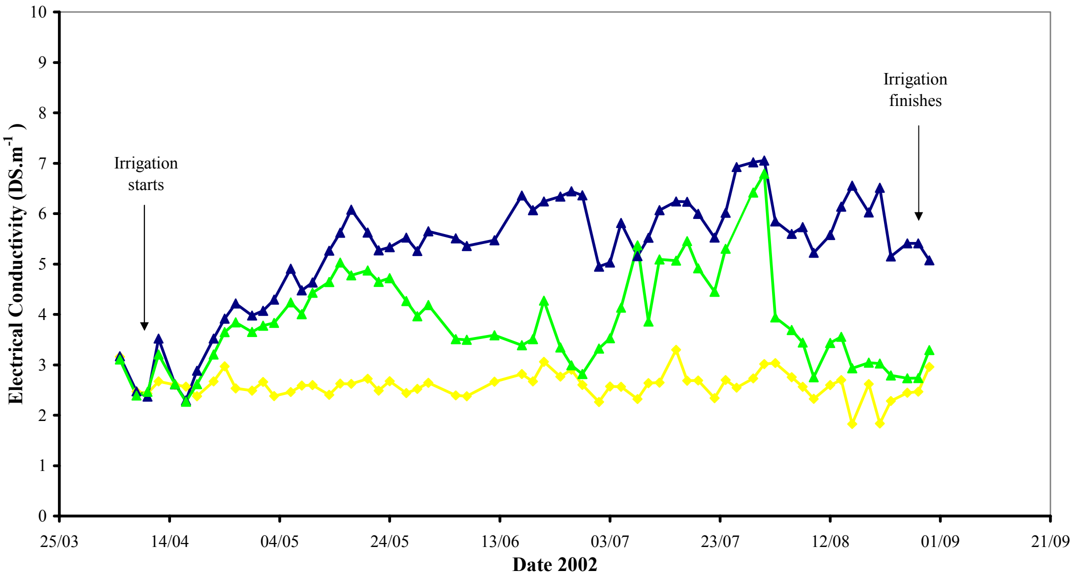

Figure 3.4: Average EC over the 2002 season recorded from the 05/04/02 until the 30/08/02 with the ECK-1 the water only irrigation treatment is indicated by the yellow treatment, the leahate only treatment is indicated by the blue treatment and the cyclical leachate:water irrigation treatment is indicated by the green series.

However flushing with bore water significantly reduces the EC and if flushed for long enough time then the EC of the top 0.1 m can return to the baseline EC recorded in the control treatment. The control treatment remains at a typical EC of 2.7 DS.m^{-1} throughout the experimental season (SE mean = 0.05). The soil pore water EC data for 2002 demonstrates the same pattern as 2001, consequently the 2001 data is not shown.

3.5.4 Permittivity of the soil during leachate irrigation

Figure 3.5 shows more that the average permittivity over the experimental period is considerably different between treatments.

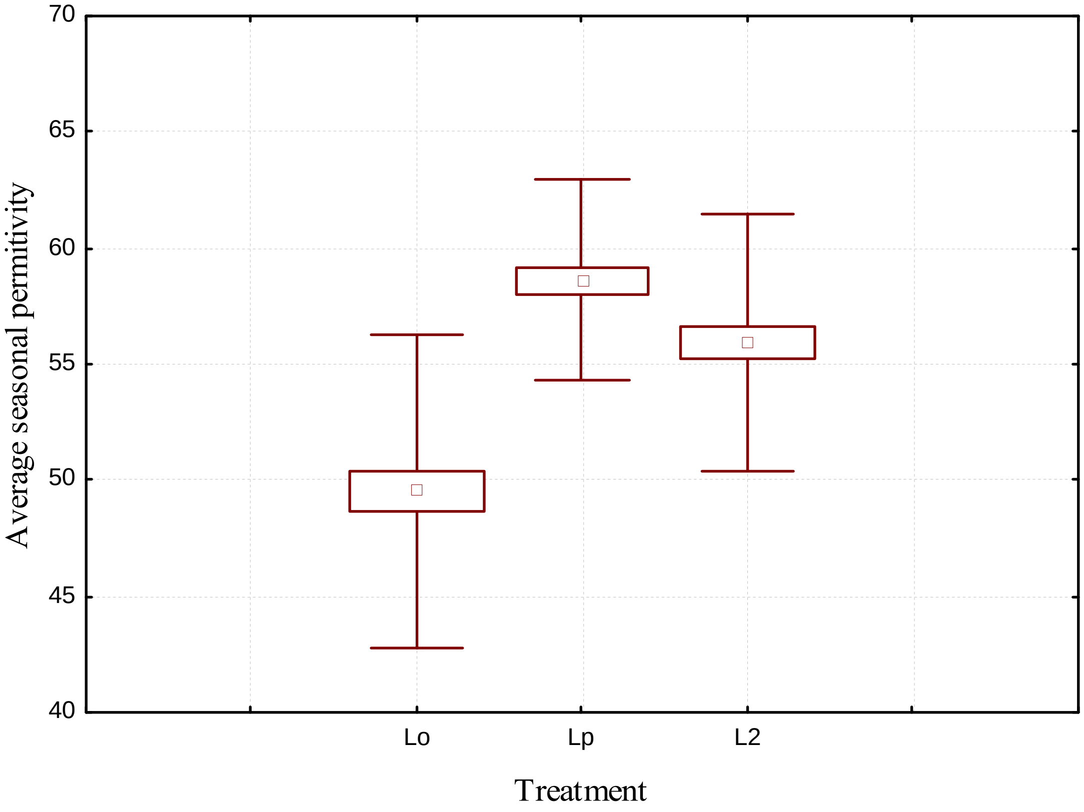

Figure 3.5: Average permittivity sampled every 2-3 days from the 05/04/02 until the 30/08/02 using the ECK-1 where Lo is the water only irrigation treatment, Lp is the persistant leachate treatment and L2 is the cyclical leachate:water treatment. Data shown are mean, standard error of the mean (box) and standard deviation (whisker).

The water only irriagttion treatment has a lower average permittivity over the experimental period than the leachate treatments; in particular the persitant leachate treatment has the highest permittivity over the experimental period between the two leachate irrigation treatments.

3.5.5 Soil temperature over the irrigation period

The measured lysimeter temperature increased as the experimental period progresses (data not shown), but Figure 3.6 shows that there is no significant difference in temperature between treatments.

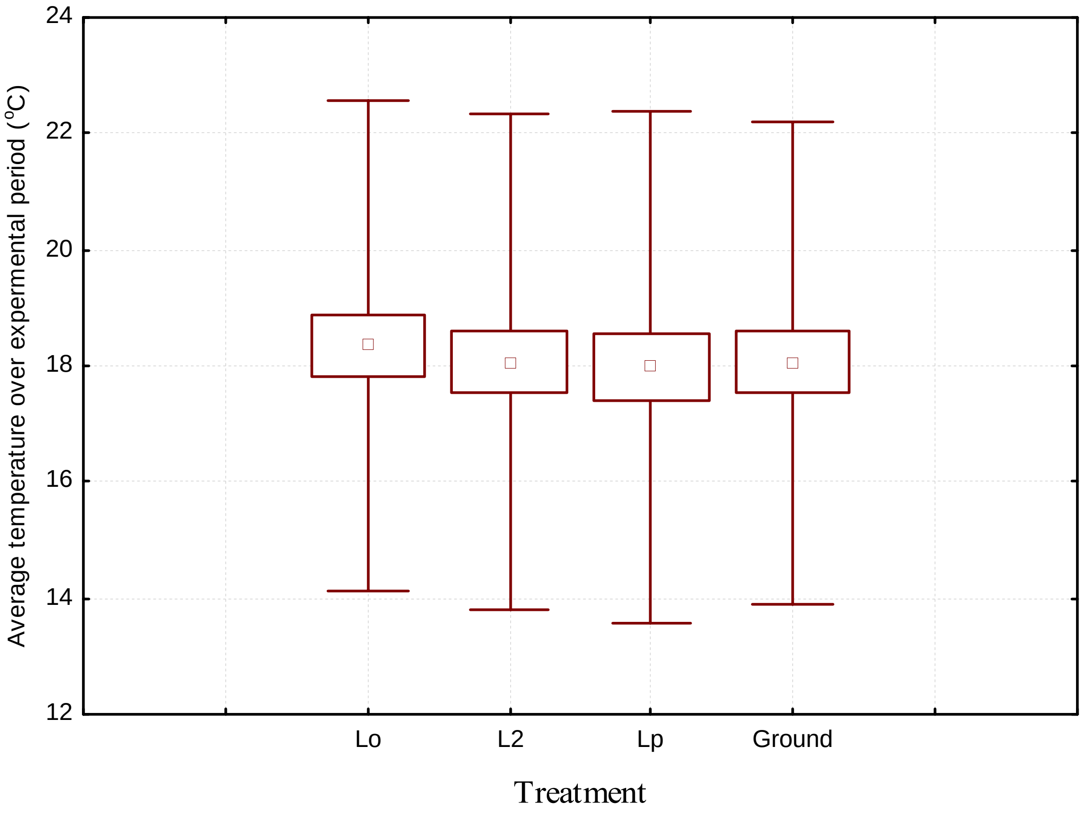

Figure 3.6: Mean soil temperature of the lysimeters and ground measured at 0.1m depth from the 05/04/02 until the 30/08/02 with the ECK-1, where Lo is the water only irrigation treatment, Lp is the persistant leachate treatment and L2 is the cyclical leachate:water treatment and ground is a spot 5 m from the experimental plot. The data shown is mean (centre), standard error of the mean (box) and standard deviation (whisker).

Furthermore, Figure 3.6 shows that there is no difference between the ground temperature and the temperature of the lysimeters.

3.5.6 EC and leachate salt application

Figure 3.7 shows no particular trend between the instantaneous measurement of EC and cumulative salt loading (specifically ammonium and chloride) for any of the cyclical leachate and water irrigation treatments.

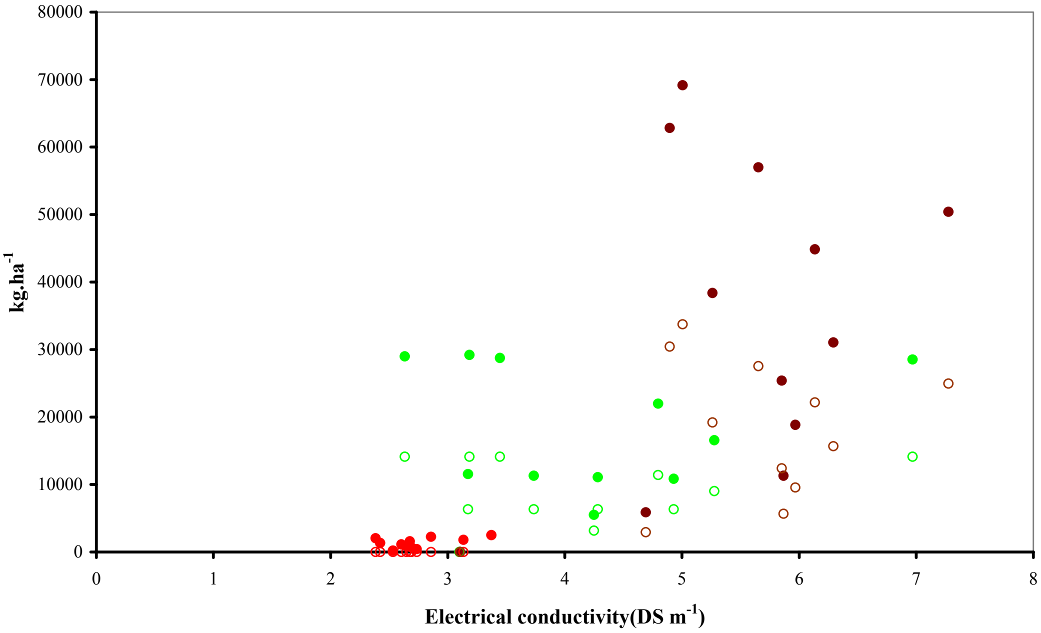

Figure 3.7: Instantaneous measurement of Oxford clay EC using the ECK-1 and cumulative chloride (filled data points) and ammonium (open data points) application under cyclical leachate:water irrigation (green) water only irrigation (red) and leachate only irrigation (brown).

Figure 3.8 however, which incorporates a time element into the X axis of conductivity measurement, demonstrates a much more positive correlation with cumulative salt application. The salt addition to the persiatnt and cyclical leachate irrigation treatments follows a linear trend for both chloride ($R^2 = 0.961$) and ammonium ($R^2 = 0.964$), the water only irrigation treatment however; contributes to a less extent to the linear trends when compared with the leachate treatments.

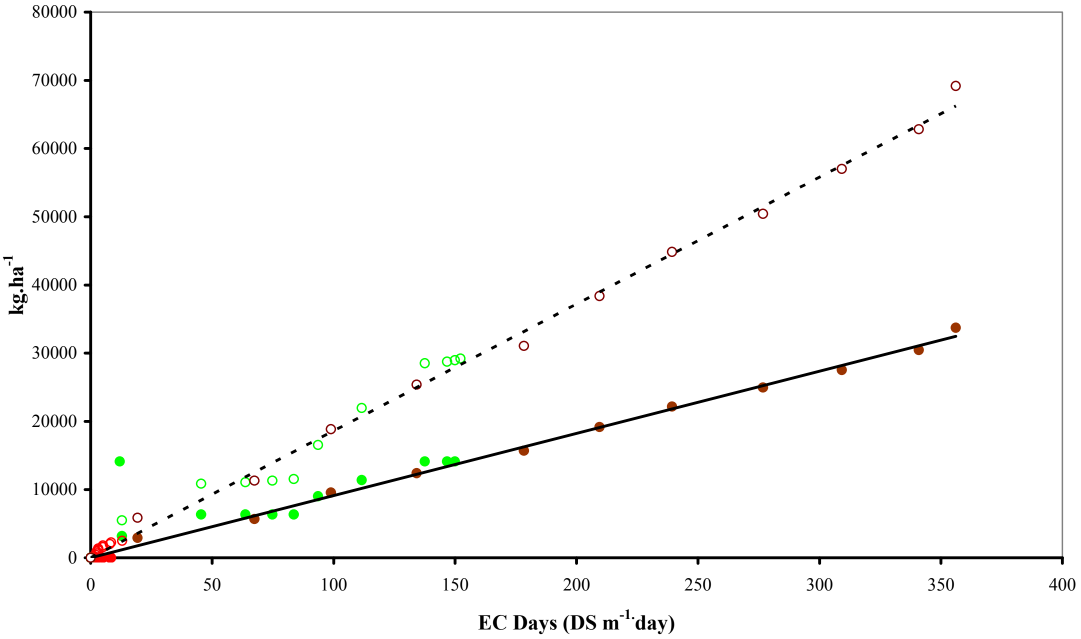

Figure 3.8: The cumulative application of chloride and ammonium and EC days, the dashed line indicates the linear relationship between chloride application and EC days for persistant (brown series) and cyclical (green series) leachate irrigation treatments, and the bold line indicates the relationship with ammonium application and EC days for persistant and cyclical leachate irrigation treatments.

3.5.7 EC days and matric potential

When the instantaneous measurement of EC is converted to EC days and plotted with plant equilibrium water potential a positive correlation following an exponential trend. There is a slight difference in data trend between 2001 and 2002. When the two data sets are combined the correlation coefficient (R^2 = 0.899) is greater than the data for a single year.

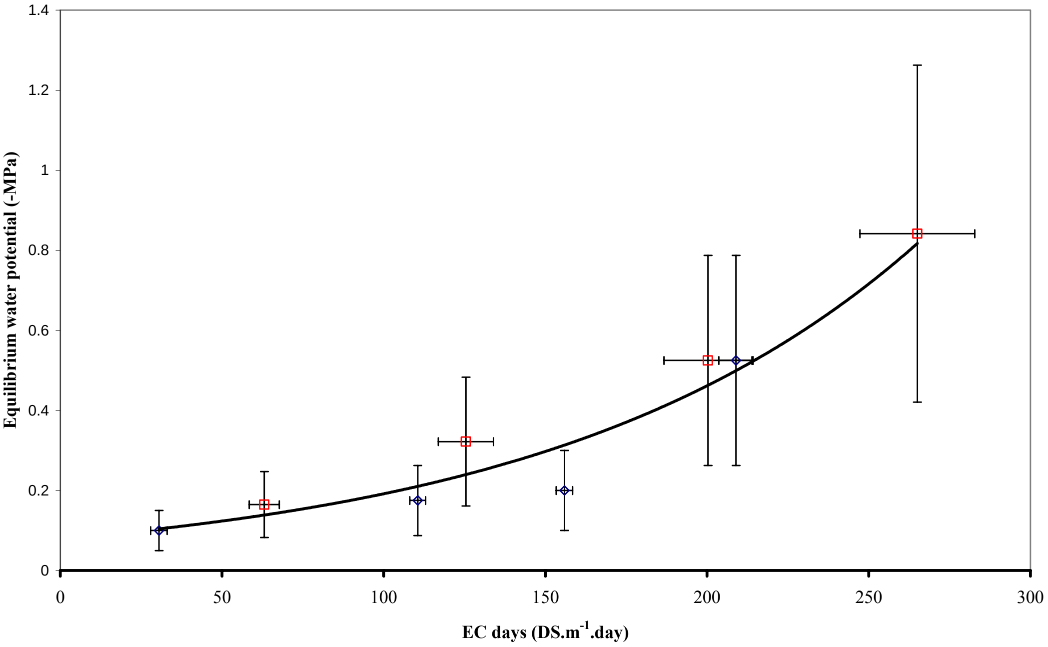

Figure 3.9: EC days and equilibrium water potential for measurements taken in 2001 (blue) and 2002 (red). The trendline follows an exponential relationship and the error bars are SE mean.

3.6 Discussion

Oxford clay has a higher baseline EC than that of agricultural soils sampled (see Table 3.3). This could be because clays have a crystalline structure with numerous imperfections, thus, creating a large negatively charged surface area (McNeil, 1980). This negative charge (also known as surface conductance) attracts cations such as calcium (Ca^{2+}) and sodium (Na^+) which are loosely held to the clay particle surface by the charge (McNeil 1980). In the presence of water an ionic halo forms around the clay particle (Keller and Frischknecht, 1966) and the cations held by charge go into suspension (McNeil, 1980). For this reason clay soils exhibit a net higher EC when compared with other soils and this may explain the EC of the extract in section 3.5.1 being higher than the leachate EC. From Table 3.1 it is evident that Oxford clay falls into the category of slightly saline (Using both the ECK-1 and CMP-11 probes see Figure 3.3).

Curiously the EC of the persistant leachate irrigation treatment plateaus at 6 DS.m^{-1} despite continous salt loading (see Figure 3.3). This was unexpected because the baseline EC for Oxford clay is approximately 3 DS.m^{-1} (See Table 3.3 and Figure 3.3) and the average EC of the leachate applied is approximately 12 DS.m^{-1} therefore one would expect persistent application of leachate to increase the pore water EC to that of the leachate applied i.e. 14-15 DS.m^{-1}, similar to the EC recorded in Figure 3.3 for the 5th solution. A similar trend was also recorded for the treeless control experiment (data not shown) although at the time the plateau was attributed to the length of the experiment. Possibly atmospheric precipitation could influence the lysimeter EC but extended periods without rain were recorded during the 2002 season with no difference in the EC recorded.

There are explanations for this observation some of the salts may have precipitated in the soil which results in a constant EC, although this is most likely to affect ammonium compounds. Oxford clay is a slightly saline soil (see Table 3.1) and leachate irrigation involves large quantities of chloride to be applied, therefore the influence of ammonium precipitation is unlikely to account for such an outstanding trend in EC. Additionally, infiltration of salts into the inner part or out of the lysimeter could account for the plateau, possibly the ionic halo around a clay particle could become saturated and therefore further application of ions would be left to percolated down into the lysimeter due to gravitational force. Alternatively, the clay could conceal the effect of salt application on soil EC through its inherent “buffering capacity”, as previously mentioned Oxford clay soil is a marine clay composed if illite and smectite clays. The smectite clays (which are negatively charged) are capable of adsorbing chloride ions (which are also negatively charged) through intermediate polyvalent cation such as calcium or water which is capable of satisfying one negative charge of the clay and one of a free cation, this reportedly is a common mechanism for the adsorption of organic matter to clays (which also has an overall negative charge) (A Gregory pers. com. 2003). This mechanism could be accentuated by the illite smectite layering which in the presence of water may be liable to expand and expose more surface area for polyvalent charges to attach themselves. However the sustainability of such a process requires research and further investigation.

Martin *et al.* (2002) noted that stem flow contributes significantly to the delivery of rainwater. It is intuitive that the size of a tree is related to how well coupled it is to the atmosphere. The leachate only irrigation treatment, which had significantly amplified dry weight and leaf area (see chapter 4) could possibly dilute the elevated osmotic potential through rainwater addition delivered by stem flow. However, prolonged dry periods were recorded in the 2002 season with no significant increase in measured EC, and the lysimeters although at 1 m^2 spacing were relatively uncoupled to the environment. Finally the EC was measured exclusively with the Sigmaprobe ECK-1; therefore the plateauing could be a product of the probes inability to record above 6 $DS.m^{-1}$ in soil. This hypothesis was disproved by the experiment to compare methods detailed in Section 3.5.1. Although an overall significant ($p < 0.001$) under recording relative to the standard method was apparent, the probe was able to record ECs substantially greater than 6 $DS.m^{-1}$. However when the extract EC is measured no significant difference between the measured EC using the CMP-11 and ECK-1 is apparent ($p = 0.1803$). Indicating that the soil has a buffering capacity possibly a product of the soil matrix or alternatively the free carbonates in the soil.

The plateauing trend was accommodated by incorporating a temporal factor into EC data used. This resulted in an improved correlation between EC and both salt loading (see Figure 3.8) and soil matric potential (see Figure 3.9). The EC days equation may also be applied to any soil as it incorporates a baseline EC and therefore provides a means to compare salt application to soils. Despite the suitability of EC for this purpose it is noteworthy that EC is relatively more spatially variable compared to temporal variations (Hartsock *et al.,* 2002). Future studies might not require such accurate monitoring of irrigation delivery and therefore investigations into the different protocols of EC measurement are recommended.

In addition to EC, permittivity and soil temperature were also measured concurrently. Treatments where leachate was a component of the total irrigation had an average season permittivity is higher than the control (water only irrigation) treatment despite being comparable at the beginning of the irrigation period (data not shown). Permittivity is correlated to water content and therefore one of the reasons why permittivity is higher could be because leachate irrigation invoked saline conditions which inhibit consumptive water use (De Jong *et al*. 1979 and Nelson and Ham (2000). The apparent increase in water content of the soil highlights the problem of irrigating the same depth for treatments which are stressed and the control and not being sufficiently sensitive to the decline in water use. Water logging of the lysimeters may explain the deleterious response persistent leachate irrigation had upon willow production. Permittivity could also be attributed to an increase in sodicity; elevated sodium concentrations (see Table 2.1) may instigate a breakdown of the clay structure which may accentuate water logged conditions by inhibition of water movement due to the lack of physical structure, although the majority of the clay structure is lost under compaction. Temperature, on the other hand, shows no significant difference regardless of the treatment or location of the measurement. One would expect the lysimeters to have an elevated temperature (despite their large thermal mass) relative to the soil they stand on because of exposure to ambient conditions. However it is likely that the aerial biomass provided some degree of shading, alternatively the ground sample (which was obtained from a point five metres outside the plot) was unrepresentative of "natural" conditions due to compaction and vegetative thinning from heavy machinery traffic. Landfill caps may experience elevated soil temperature due to sub surface decomposition (up to 40 ^{o}C, Lefebvre *et al.,* 2004), elevated soil temperature has been shown to alter root respiration (Boone *et al.,* 1998) and aerial gas exchange (Ziska, 1998 and Xu and Huang, 2000), moreover elevated root zone temperature may increase nutrient uptake (Taiz and Zeiger, 1998) and increase denitrification (Ilies and Mavinic, 2001). Therefore, what at first seems like a circumvention of a potential pitfall of a lysimeter based study now appears like a limitation of the study which possibly could be incorporated into further investigations.

3.7 Conclusions

Oxford clay soil can be classified as a "slightly saline" soil. Irrigation with leachate resulted in an increase in the soil water EC and irrigation with water resulted no significant difference in soil water EC unless it followed a period of leachate irrigation, then irrigation with water would result in a decrease in soil water EC.

Persistent leachate irrigation resulted in a soil water EC which plateaued well below the EC of the leachate applied. The incorporation of a temporal factor in the measurement of EC (EC days) simply and reliably records chloride and ammonium addition and soil matric potential.

4 Morphological implications of leachate irrigation

4.1 Introduction

The maintenance of healthy plant growth is of paramount importance for successful establishment, functioning and sustainability of a landfill leachate attenuation system. Leaf area in particular, is linked to biomass and total evapo-transpiration which is especially important for the attenuation of landfill leachate volume and soil water status.

4.2 Literature review of the morphological implications of leachate irrigation on *Salix viminalis*

4.2.1 Plant biomass and response to leachate application

Production of biomass is correlated with the application of nitrogenous compounds in a number of species for example; wheat (Latiri-Soukii *et al.,* 1998), willow "Tora" hybrid (Weih, 2001) and cabbage (Wong and Leung, 1989). Conversely, saline conditions have been demonstrated to decrease biomass for certain non-halophytic species. For example De Pascale and Barberi (1997) recorded a 50% reduction in biomass of broadbeans under exposure to a soil EC of 5 $DS.m^{-1}$.

The impact of leachate on the biomass of coppiced trees has been investigated by a number of authors (Ettala, 1988, Wong and Leung, 1989 and WRC 2002). Ettala (1988) found an amplification of 215% in *Salix aquatica* biomass compared with controls grown on landfill sites. Wong and Leung (1989) found, however, that the aerial portion of *Brassica parachenisis* responded positively to leachate application, whilst the below ground portion was inhibited. Both the above and below ground portions of *Acacia confusa* was inhibited by leachate application. WRC (2002) found 22-27% stimulation in biomass of leachate irrigated willow *sp.* relative to controls when grown on a landfill cap restored for agricultural production. The leachates applied in the above studies had a lower EC than the one used in this study and the irrigation depth was much lower than the one adopted in this study.

Previous studies have not involved regular analysis of growth rate for the duration of leachate irrigation but rather, analysis at the conclusion of the period of irrigation. For effective management of a landfill leachate attenuation system data is needed on the relationship between growth and osmotic potential.

4.2.2 Leaf area response to leachate application

Numerous authors have noted that water stress affects cell expansion and cell division (e.g. Taiz and Zieger, 1998 and Levitt, 1980). It follows that these restrictions will manifest themselves at a foliar level. Therefore, if leaf cell growth and division is inhibited then leaf size and shape will be affected. Souch (1996) noted that individual leaf area of poplar hybrids was inhibited by water stress. Taiz and Zieger (1998) state that leaf area index (which is a function of leaf number) is diminished by water stress, and decreasing leaf area can be considered an early *adaptive* response to water stress. Leaf area has been shown to be very sensitive to EC (more so than biomass) in a number of studies with a variety of plant species e.g. in broadbean (Kateriji *et al.,* 1992) in pea (De Pascale and Barberi, 1996), in aubergine (Ruggiero *et al.,* 1994) and lettuce (De Pascale and Barberi, 1995) all cited in De Pascale and Barberi (1997).

Conversely the application of nitrogenous compounds has been shown to increase leaf area Latiri-Souki (1998).

4.2.3 Estimates of biomass and productivity

There is no standard method of measuring biomass of rapidly growing coppiced trees. Ettala (1988) and Cureton *et al.* (1991) used height to measure the biomass, whereas Maurice *et al.* (1999) and Martin *et al.* (2001) found that measurement of stem basal area (coupled with the incorporation of a retrospectively determined allometric relationship between diameter and dry weight) was a more favourable measurement.

Leaf area is directly related to light interception, whole plant transpiration and carbon uptake, and therefore is considered to be the most important single determinant of plant productivity (Verwijst and Wen, 1996, citing Linder, 1985). Reliable estimations of leaf area are tedious and labour intensive and so indirect measurements of leaf area via light interception are sometimes adopted e.g. Martin *et al.* (2002). However in an enclosed lysimeter study neighbouring trees may impose upon the measured intercepted light of a single tree. Therefore, a more laborious methodology, where the total number of leaves is counted and individual leaf area is calculated to produce an estimation of leaf area is more appropriate.

The estimation of individual leaf area from a non-destructive measurement of length and width has been adopted by a number of authors e.g. Verwijst and Wen (1996) and Ramkhelawan and Braithwaite (1990). The conversion of linear measurements to individual leaf area is species specific. Some authors have found that that the measurement of width is sufficient e.g. Ramkhelawan and Braithwaite (1990) and Soule and Malcolm (1970). Others e.g. Robbins and Pharr (1987) found that both length and width was necessary. P Martin (pers. com. 2001) satisfactorily adopted a linear model even though Verwijst and Wen (1996) found that a non linear model was more suitable for the estimation of individual leaf area of *Salix* leaves.

P. Martin and I. Seymour (pers. com. 2001) identified clustering of the *Salix viminalis* leaves, and both have proposed methods to accommodate this. Martin increased the distance between lysimeter grown trees and adopted the use of a solar stick for measurement of intercepted light. I Seymour adopted a destructive analysis of randomly selected leaves within the cluster and coupled with the length of the cluster calculate leaf area.

4.2.4 Qualitative measurements

A number of qualitative measurements were employed throughout both the 2001 and 2002 experimental season; these include incidence of flowering, proliferation of side branches and degree of epinastic response.

4.3 Objectives

The following objectives were fulfilled by the outlined experiments;

- To derive an allometric relationship between measured stem basal area and dry weight of *Salix viminalis*,
- To investigate the temporal changes in biomass accumulation of *Salix viminalis* in response to periodic and persistent leachate irrigation,
- To quantify the changes in leaf area of *Salix viminalis* and the modulating components of leaf area with leachate irrigation and flushing.

4.4 Method

4.4.1 Stem basal area/dry weight relationship

At the end of the experimental period (between 1st Sept - 30th Sept 2002) 15 three year old S*alix viminalis* trees which had previously been grown on Oxford clay soil and irrigated with either leachate or water were harvested. The stem basal area was measured with electronic callipers perpendicular to the branch axis within the bottom 3 cm of the branch. Then the branches where chopped up and stored in paper envelopes until drying. Drying took place between the 7th - 21st Jan 2003, the oven was set to 105 ^{o}C and the shoots were left there for >4 days, (Lindegaard *et al.,* 2001). A selection of a few samples were weighed one day and again the next if the weights were within -/+ 5% of each other then the rest of the samples were weighed.

4.4.2 Stem dry weight and growth rate

The measurement of stem size borrowed heavily from the methods of Maurice *et al.* (1999), who measured diameter rather than height (c.f. Ettala, 1988 and Cureton *et al.,* 1991). Stem diameter was measured perpendicular to the axis of the branch within the bottom 50 mm. Measurements were taken at monthly (2001) and 5-week intervals (2002) using electronic callipers (Camlab, Cambridge). The branch was assumed to be circular and every stem within the plot was sampled. The relationship between measured girth and biomass was calculated using the relationship between the natural logarithm (Ln) of stem basal area (SBA) and the Ln of dry weight as determined by destructive harvesting. The branch biomass was calculated retrospectively using an equation derived from the destructive harvest where;

$$\text{Ln DW} = 0.9872 \text{ x } (\text{Ln SBA}) - 0.6093)$$

Equation 4.1: The equation used to calculate the dry weight of a stem from the stem basal area where; DW = Dry weight (g) and SBA = Stem basal area (mm^2).

4.4.3 Leaf area estimation method

A stratified sampling strategy was adopted to select 15 stems in 2002 season. Every 20th leaf was systematically measured for width (at the widest point on the leaf) and length (from base of lamina to leaf tip) and the total number of leaves was recorded. The individual leaf area was calculated using Equation 4.2;

$$\text{Individual leaf area} = (L \text{ x } W) \text{ x } 0.729$$

Equation 4.2: Calculation of individual leaf area (pers com P Martin pers. com 2002) for expression in mm^2 where L = leaf length and W = leaf width.

Total branch leaf area was calculated by averaging the individual leaf areas sampled then multiplying the average by the total number of leaves. Leaf area values were then plotted against the stem diameter in order to generate an R^2 correlation coefficient and to produce a descriptive equation describing a possible allometric relationship between girth and leaf area. The allometric equation was then applied to the diameter measurements of all branches measured within 4 days to produce the whole tree leaf area.

Figure 4.1: Branches of a leachate irrigation only treatment tree showing the overproduction of leaves even in the lower zone of the tree.

Because the R^2 values generated from applying the leaf area to the girth were highly variable ($R^2 = 0.1537 - 0.93$) extrapolation using the equations is flawed (see also

Clendenen, 1996). Therefore, leaf area per unit stem basal area was used as a conversion factor to manipulate the stem basal area data to meaningful leaf area data.

4.5 Results

4.5.1 Stem basal area/dry weight relationship

Figure 4.2 graphically illustrates a linear allometric relationship between dry weight and stem basal area.

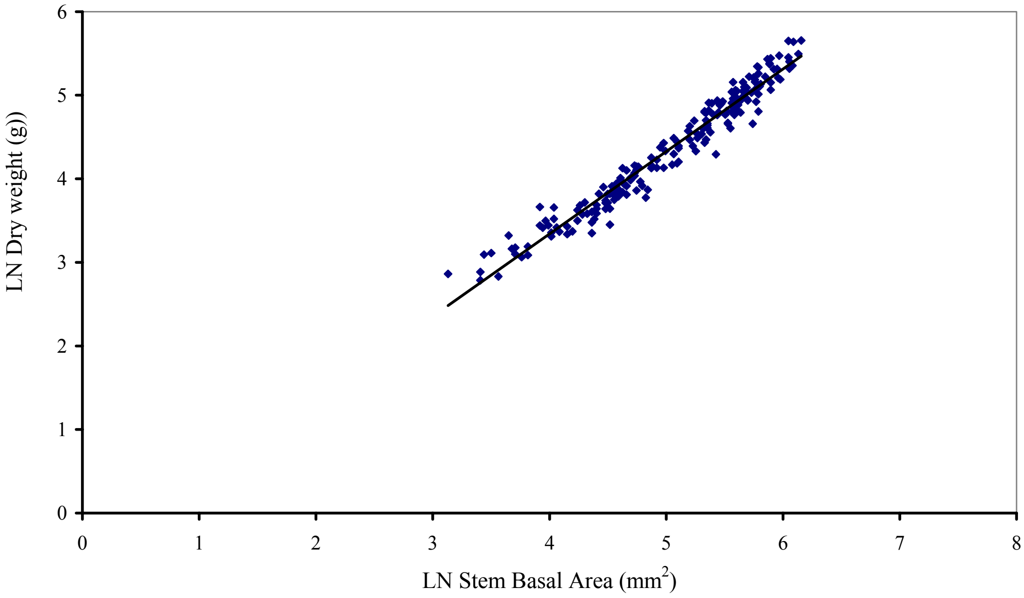

Figure 4.2: Natural logarithm of stem basal area with dry weight, each data point relates to an individual branch.

Due to improved R^2 value (0.9557) obtained when the raw data is converted through a natural logarithim the data is express as such.

4.5.2 Dry weight

Figure 4.3 shows leachate to have a stimulatory effect on dry weight of lysimeter grown *Salix viminalis*. Statistical analysis resulted in LSD between treatments of 239.3, therefore Figure 4.3 shows that there are no significant differences between leachate and water irrigation treatments dry weight at the start of the 2001 season. However in the subsequent seasons the accumulation of dry weight of the water irrigated treatment is significantly reduced relative to the leachate irrigated treatment.

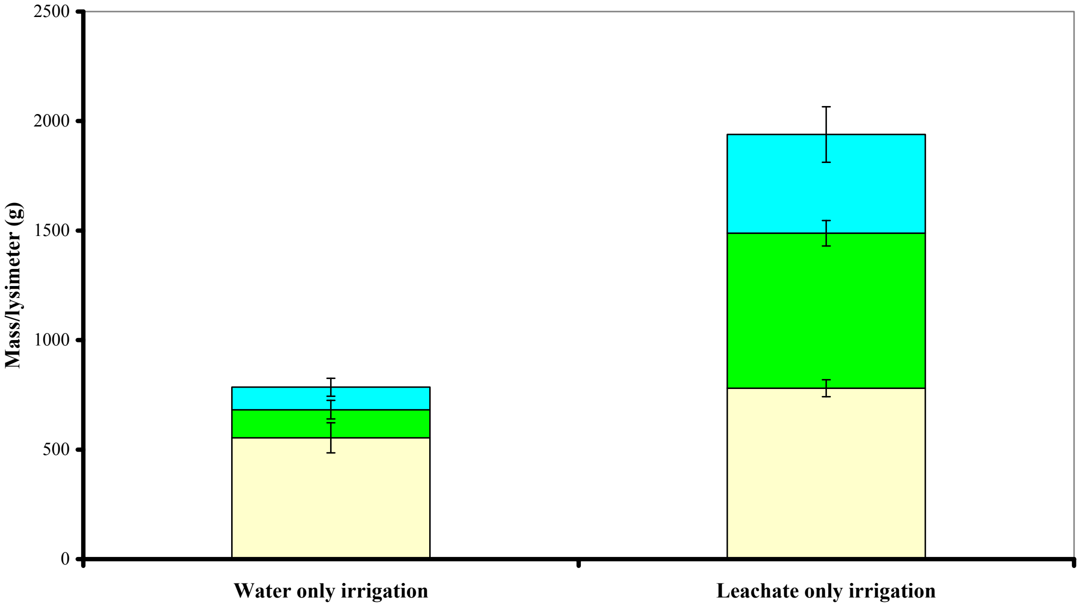

Figure 4.3: Dry weight accumulation of the water and leachate irriagted treatments over the course of the experimental period. Where the yellow portion indicates the average dry weight/lysimeter by 30/04/01, the green portions indicates the incremental accumulation of dry weight by 15/10/01 and the green portion indicates the incremental accumulation of biomass by 31/10/02, the error bars are standard error of the mean.

Ultimately the leachate only irrigation treatment amassed 236% more dry weight than the water only irrigation treatment. Analysis of the dry weight of all the treatments which received either continous or cyclical leachate irrigation resulted in no significant differences (p = 2.02) between treatments (data not shown).

4.5.3 Leaf area

Figure 4.4 shows the defoliation of leachate only and cyclical leachate:water treatments with leachate irrigation. A clear linear trend, with a high correlation coefficient ($R^2 = 0.78$) describes the progressive defoliation of *Salix viminalis* with increasing EC days.

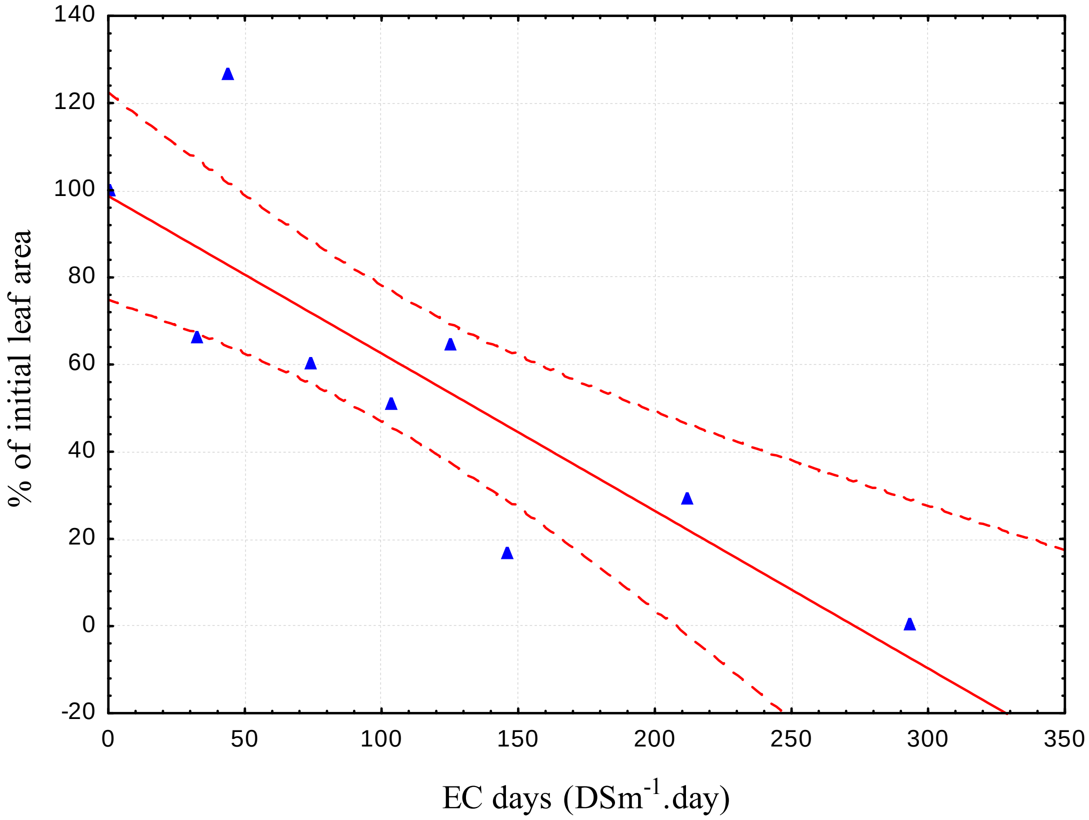

Figure 4.4: Calculated leaf area of trees expressed as a percentage of the initial leaf area the red line shows a linear fit to the data. The doted red lines indicate the 95% confidence interval boundaries.

The mode of leaf area measurement facilitates a detailed analysis of the mechanism of leaf area reduction. Figure 4.5 shows that the leachate irrigation treatment average individual leaf area didn't significantly change throughout the experimental season. There is a small difference between the months of March and April compared to the months of May and June, but this is as likely to be a result of the type of leaf sampled.

Finally there is no data presented for the month of August because total or near total defoliation was evident in leachate irrigation treatment by this point in the season.

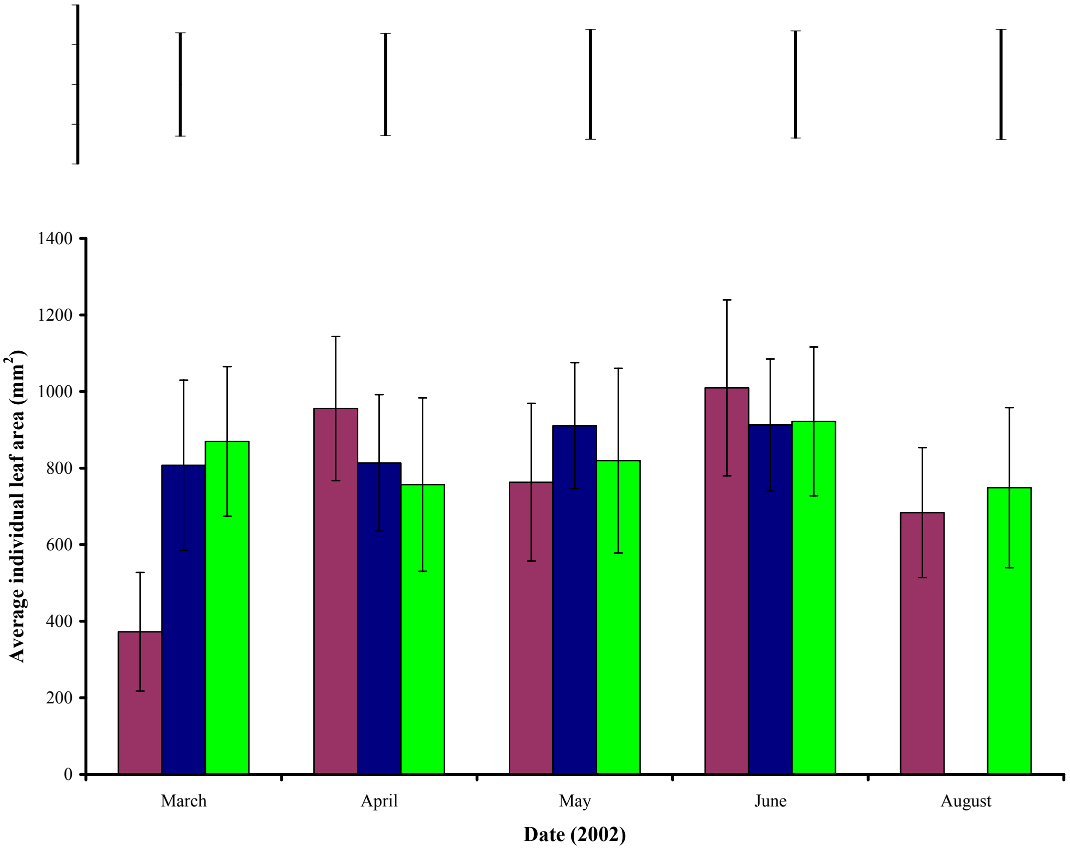

Figure 4.5, Average individual leaf area of persistant leachate (blue), water only (purple) and cyclical water:leachate irrigation treatments measured on the weeks commencing 25/03, 22/04, 20/05, 24/06 and 19/08. LSDs are above the graph these were calculated per sample date and the error bars are standard error of the mean.

Figure 4.5 shows that there is significant differences between the water irrigated leachate irriagated treatments in the month of March. After which the individual leaf area is not significantly different between treatments throughout the season, until the defoliation of the leachate only irrigation treatment. Aside from the defoliation of leachate only irrigation treatment in August there are no significant differences (p = 0.26) between the dates of sampling.

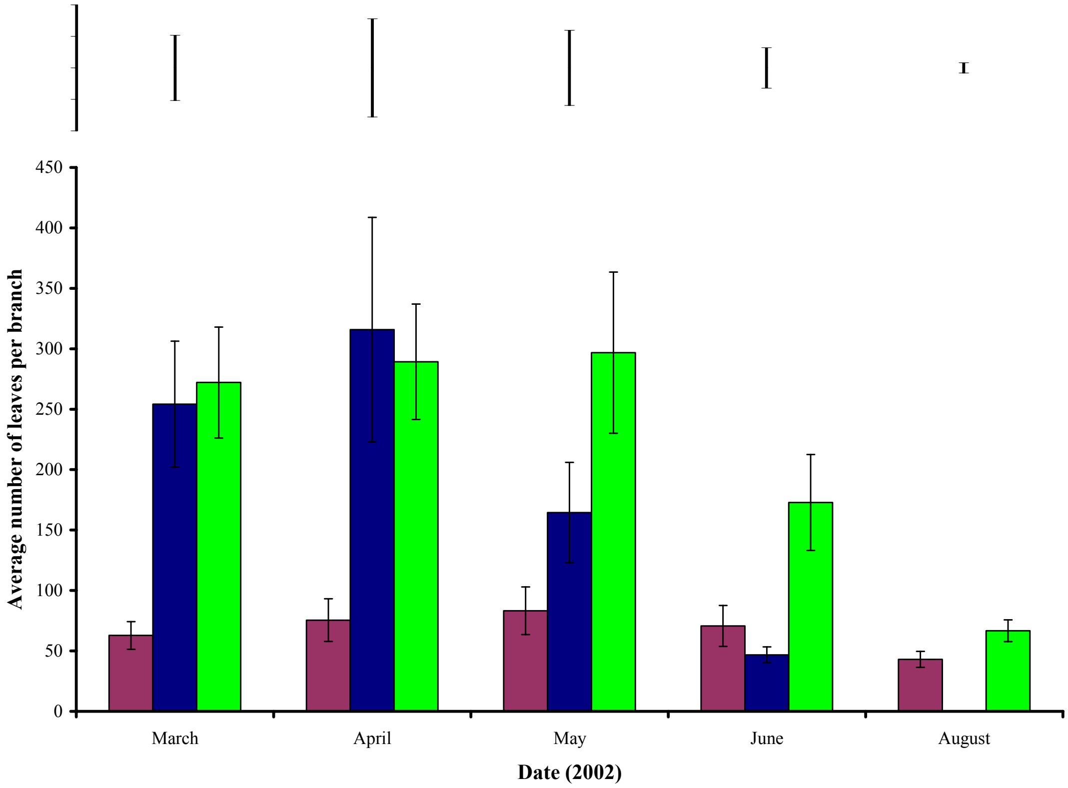

Figure 4.6: Average number of leaves of persistant leachate (blue), water only (purple) and cyclical water:leachate irrigation treatments measured on the weeks commencing 25/03, 22/04, 20/05, 24/06 and 19/08. LSDs are above the graph these were calculated per sample date and the error bars are standard error of the mean.

Leaf number however is significantly influenced by leachate application. Firstly there is no significant difference between the number of leaves of the persistant and cyclical leachate irrigation treatments in March and April but from May onwards there is significant differences until the eventual defoliation of the leachate only irrigation treatment (see Figure 4.6). Furthermore there are significant differences ($p < 0.001$) between the leachate treatments and the control on all dates of sampling. In August there is no significant difference between cyclical leachate:water and water only irrigation treatment whereas two months earlier there were. By the end of the experimental period the cyclical leachate:water treatment has 20% of its original leaf number.

4.6 Discussion

Critical analysis of the treatment differences in dry weight and growth rate first requires some discussion of the methodology and retrospectively applied equations. Other authors have found that an allometric relationship between stem diameter and dry weight of coppiced *Salix* species to be a functional non destructive tool for biomass measurement (Proe *et al.*, 1999 and Martin *et al.,* 2002), although the technique has not been applied in all projects where the biomass of coppiced *Salix* has featured in the experimental results due to a variety of reasons. In this study, a non destructive methodology was desirable because of the limited amount of plant material and the desire to repeat measurements on the same sample population; consequently a retrospectively produced equation was applied. Typically one would assess the suitability of an allometric relationship from the R^2 values obtained. The equation to describe the relationship using the natural logarithms of measured dry weight and converted stem basal area was deemed to be most reliable method of obtaining regular measurements of above ground plant biomass. However there is also the potential for errors in the measurement of diameter; one assumes that the branches are perfectly round; moreover it is virtually impossible to return to the same position on each branch. These two factors may explain the occasional negative values and errors observed in the translated data. However the importance of these negative values was considered negligible due to the volume of data collected.

In the experimental plot every tree was sampled and a clear stimulation of shoot biomass was recorded relative to the water only irrigation treatment. At the end of the experimental period a significant ($p > 0.001$) two fold stimulation of shoot biomass was recorded. This is comparable to the 216% increase in dry weight recorded by

Ettala (1988), although ten times more than the 22-27% increase in dry weight recorded by the WRC (2002) project. One reason for the different responses in amplification could be the substrate the plants were grown in. WRC (2002) grew willow in a landfill cap restored to agriculturally suitable standards this study like that of Ettala (1988) grew willow in landfill cap liner. In nutrient impoverished substrate the difference between control and treatment is accentuated, especially as the water only irrigation treatment dry weight accumulation was stunted from the 2001 season onwards see **Figure 4.3**. This concurs with work done by Martin *et al*. (2002) who concluded that nutrient status is more important than bulk density on the aerial portions of willow growing in Oxford clay. Figure 4.3 illustrates that the water only irrigation treatment accumulated an insignificant amount of dry weight in 2001 and 2002, which maybe indicative of nutrient impoverishment. However one of the most interesting findings of this experiment is that there were no significant differences between the accumulated biomass of the leachate treatments, despite the cyclical irrigation schedules delivering 50% and 75% leachate relative to the persistant leachate treatment. This indicates that either too much or too little leachate was applied for optimum biomass production. Therefore, future may want to address this gap in knowledge; however it is pertinent that the revenue generated by a landfill leachate attenuation system would be primarily from the treatment of leachate.

Numerous authors (e.g. Monteith, 1977 and Verwijst and Wen, 1996) highlight the correlation between leaf area and plant productivity. In this study an estimate of measured leaf area was recorded rather than intercepted light (often used as a surrogate for leaf area) because of the possibility of mutual shading between lysimeter. The application of leachate created saline conditions (see Section 3.5.3) which resulted in leaf droop (not quantified) this in turn would reduce the amount of intercepted light and reduce the fixed CO_2. Therefore, the estimate of leaf area is somewhat flawed insofar as it doesn't account for the intercepted light. It is noteworthy that amplified biomass and leaf area may result in increased rainfall capture and stem-flow which supplements irrigation delivery and may alleviate soil matric potential stress.

The elevated leaf area may not exclusively control the stimulation of growth rate. Abundant nitrogen input is considered to reduce mycorrhizal associations (Bethanfalvay *et al.,* 1997 and Smith and Read, 1997) however counter to conventional thinking landfill leachate has been shown to stimulate mycorrhizal populations (Baum *et al* 2002, Maurice *et al.,* 1999) which would significantly contribute to the absorption of essential plant nutrients. Furthermore, assimilation of nitrogen compounds will result in increased biomass and increased foliar RUBISCO content (Raven and Sprent, 1993) may alleviate the adverse effects of water deficits on biomass accumulation.

No modulation of individual leaf area was apparent (see Figure 4.5) this is somewhat surprising as it is counter to the effect of water stress on *Populus* sp. (Souch, 1996). The reduction in leaf area relative to the original leaf area is modulated by defoliation. It is likely that the trees overproduced leaves at the beginning of the season (persistant leachate treatment has a leaf area of 7.3 m^2 and water only irrigatioin treatment has 0.51 m^2) and, therefore, because of shading or energy demand a proportion of these leaves would be shed naturally. Yet irrigation with leachate and the creation of saline conditions appears to have accelerated this process.

Figure 4.7: Three example trees from each of the treatments, from left to right: water only, cyclical leachate:water and leachate only irrigation treatments, picture was taken in November 2002.

The proliferation of leaves at the beginning of the season is illustrated further when one considers that the maximum leaf area should occur in August for this species (Souch, 1996). The over production and subsequent defoliation of leaves may be an important factor in the consideration of the viability of a landfill leachate surface recycling system, on one hand the leaf material may reduce erosion and contribute to humus produced, on the other chloride accumulated in the leaves may either inhibit humification or be incorporated into the substrate resulting in even more saline conditions for plant growth and probably add to chloride washout.

The eventual defoliation is encouraging because full defoliation did not occur until an EC days of 200 DS.m^{-1}.day was exceeded (see Figure 4.4). As previously mentioned leaf area has been reported to be highly sensitive to EC for a number of species (Kateriji *et al.,* 1992, and De Pascale and Barbieri, 1997). In this study a good linear fit ($R^2 = 0.74$) was found between EC days and leaf area. The persistence of leaf area is remarkable in comparison with the findings of Cureton *et al.* (1991) who observed weeping willow defoliation in response to an irrigation schedule described as "heavily excessive". In fact the chloride concentrations were 1991 μg.l^{-1} and the irrigation rate was 25 mm twice weekly, so effectively the irrigation depth was 14% the depth of this experiment and the chloride concentration was 1000 times weaker. This is probably a product of both the relative tolerance of the experimental species to chloride and the nitrogen loading upon the lysimeters and the fact that Cureton *et al.* (1991) re-circulated the leachate whereas in this experiment the lysimeters were kept free flowing. An over production of leaves at the beginning of a season is an interesting finding from the experiment. Data presented in chapter 6 demonstrates that after a period of 5 weeks non leachate irrigation most of the nitrogenous compounds are leached out of the lysimeter. Therefore, over winter one would expect a near complete removal of nitrogenous compounds consequently it is possible that the trees may be storing nutrients in the cambian as a resource for leaf emergence. Furthermore, the increased leaf numbers and possibly leaf nutrient status may contribute to predation intensity (Hartley and Jones, 1997) and could influence management strategies. However, this response would be advantageous for improving biodiversity in a landfill based leachate attenuation system.

The difference in growth rate and leaf area trends recorded in this study may be attributed to the timing of leachate irrigation. Latiri-Souki (1998) showed that nitrogen application invokes different responses in wheat dependent on the developmental stage of the plant. A similar situation may have occurred here, if leachate is applied at the early part of the season when the trees are growing (Proe *et al.,* 1999) then the nitrogenous compounds in the leachate may accentuate the growth rate directly. This may explain the difference in growth rate between the 2001 and 2002 season, with irrigation commencing four weeks earlier in 2002.

4.7 Conclusions

Relative to the water only irrigation treatment the leachate only treatment demonstrated a two fold enhancement of dry weight and a 14 fold enhancement of initial leaf area. The magnitude of difference between the treatments may be in part due to the water only treatment being subjected to nutrient deficient conditions. There was no significant difference in accumulated dry weight between the cyclical and continuous leachate treatment although there were large differences in leaf area between the cyclical and continuous leachate treatment. Cumulative EC (EC Days) was found to correspond well to total leaf area and leaf area was modulated through leaf number rather than individual leaf area. An EC days in excess of 200 DS.m^{-1}.day results in defoliation of the continuous leachate irrigation treatment, however cyclicial leachate irrigation resulted in improved foliar retention and therefore a reduction of the substrate EC may facilitate leaf area to persistence.

5 Physiological response to leachate

5.1 Introduction

Irrigation with landfill leachate results in high nitrogen and chloride loading of the substrate. Plant sequestration and osmotic changes in the substrate may influence plant metabolism. Understanding of the physiological implications of this will enable optimisation of the irrigation of landfill leachate to a soil/plant system.

5.2 Literature review of the physiological response of *Salix viminalis*

The photoreactions are governed by nitrogenous compounds namely the chlorophylls and the carboxylation enzyme RuBISCO. Increased foliar nitrogen status has been shown to increase the concentrations of RuBISCO (Raven and Sprent, 1993). Furthermore, chlorophyll fluorescence parameters have been used successfully to monitor nitrogen fertigation (Peterson *et al.,* 2002).

It is well documented that stomatal respond to stress (e.g. Levitt, 1980), even more so for an isohydric species in which stomatal control is integral to prevent desiccation. The mechanism by which stomata control their aperture is discussed thoroughly by Taiz and Zeiger (1998), and the biophysical/chemical signal related to stomatal sensitivity is likely to be related to the hormone ABA (Blackman and Davies, 1985). Consequently stomatal control over gas exchange during stress is an important response with implications for productivity and water use.

The degree of carboxylation is reduced by stomatal aperture or impairment of RuBISCO function (Taiz and Zeiger, 1998). Numerous stresses have been demonstrated to impair the activity and performance of RuBISCO e.g. salt stress, (Pinheiro *et al.,* 2001 and Tattini *et al.,* 2002) freezing, (Guy, 1990, cited in Taiz and Zeiger, 1998) and drought stress, (Ogren, 1988a, Delphine *et al.,* 1999, Tattini, *et al.,* 2002, Sifola and Postiglione 2002, Loreto, *et al.,* 2003). All result in a suppression of carboxylation. Either a decrease in epidermal conductance or a decrease in mesophyll conductance may suppress carboxylation (O'Toole *et al.,* 1976). A reduction in mesophyll conductance can be attributed to simple desiccation and shrinkage of cells, which would impair carboxylation through a reduction of surface area for CO_2 diffusion within the intercellular spaces.

However, a reduction in carboxylation could be due to factors other than those associated with RuBISCO performance. ATP and NADPH are necessary for the Calvin-Benson cycle to function, an impairment of the electron transfer and ATP coupling has been recorded by Tezara *et al.* (1999). Furthermore the initial acquisition of light energy and transference to the Calvin-Benson cycle can be impaired by stress (Keck and Boyer, 1974, Bjorkman and Powles, 1984).

Stress has even been recorded to change the mode of photosynthesis *Mesenbryanthemum crystallinum* demonstrates facultive CAM, which is the ability to switch from C_3 photosynthesis to CAM during periods of drought or salt stress (Hanscom and Ting, 1978, cited in Taiz and Zeiger, 1998).

Because of the nature of stress and the different experimental approaches for quantification of water stress it is unwise to make implicit comparisons between species, however the generalised trend is that perceived stress is accompanied by a reduction in photoreactions with the exception of plants which demonstrate facultive CAM.

Salix species are isohydric (Lowenstein and Pallardy, 1998) and have not been reported to demonstrate facultive CAM. Therefore it would be ineffective to utilise measurements which quantify mesophyll conductance or changes in carboxylation pathways. Techniques to quantify the energy incorporation and gas exchange are more suitable; these techniques are to be discussed in the following sections.

5.2.1 Gas analysis

Plant gas exchange has been used to quantify plant condition in response to environmental variables, including the following: seasonal variations (e.g. Williams *et al.,* 1998); response to growth regulators (e.g. Bednarz and van Iersel, 1998); CO_2 enrichment/climate change (e.g. Stlinski *et al.,* 2000); atmospheric ozone (e.g. Calatayud *et al.,* 2002); and drought and water stress (e.g. Tezara *et al.,* 2003). Typically plant gas exchange is measured using an infra red gas analyser (IRGA) where the flux of CO_2 and H_2O, relative to a known reference concentration of CO_2 and H_2O, is analysed in a sealed chamber. This may then be used to calculate parameters indicative of plant condition. Those most commonly used are: calculated CO_2 uptake (A), stomatal conductance (Gs), transpiration (T) and sub stomatal CO_2 concentration (Ci). The response of each parameter to environmental variables is species specific. However, distinct diurnal changes in gas flux are a common feature of most plant species. The diurnal variations are primarily a function of the incident radiation although these variations can be tempered by available water, seasonality and plant stress. Consequently, gas analysis is most frequently used to quantify the gas flux under ambient conditions in a comparable sample population (e.g. Oquist, 1985 and Shrive *et al.,* 1990) during a temporally comparable period e.g. whilst the sun is at its zenith (Loreto *et al.,* 2003, Sifola and Postiglione, 2002 and Delphine *et al*., 1999).

As already mentioned, gas exchange is influenced by incident radiation. Of particular importance to plants is Photosynthetically Active Radiation (PAR) in wavebands 400 – 700 nm. As light levels increase from dark to light one would expect photosynthesis to commence and CO_2 uptake to increase, initially in a linear response to increasing light levels. However, the increase in carboxylation will not be indefinitely correlated with the light intensity. An upper limit of CO_2 uptake will be reached because of photochemical limitations in the harvesting of light and transference of energy. By measuring carboxylation in response to increasing incident light levels the quantum efficiency of the photoreaction complexes can be ascertained.

Advanced techniques can also be employed to expose the performance of RuBISCO. For example, measurement of carboxylation with increasing sub-stomatal CO_2 concentrations provides insights into the velocity of RuBISCO (Km), degree of stomatal control and the rate of RuBP/RuBISCO turnover (von Caemmerer and Farquhar, 1981). Since RuBP/RuBISCO turnover is limiting at low values of sub-stomatal CO_2 concentration (Ci), the slope of the A/Ci relationship at those low values of Ci can be used to assess changes in the ability of RuBISCO to fix CO_2. This slope can therefore be called the "carboxylation efficiency". A decrease in the slope of this line is an indicator of down-regulation, in which the amount of RuBP/RuBISCO turnover is decreased relative to a control. Down-regulation is indicative of a plant under stress conditions. Therefore, investigations which compare A/Ci relationships as a result of either CO_2 enrichment or stress identify changes in the carboxylation efficiency. They determine whether these factors are influential in RuBP/RuBISCO turnover and consequently provide an insight into the mechanism of stress.

5.2.2 Fluorescence

Chlorophyll a fluorescence measurements are primarily concerned with the degree of energy incorporation. In photosynthetic systems, chlorophyll *a* molecules act as an acceptor for light energy, and chlorophyll *b*, acts as the donor of light energy. These two pigments are in close proximity and the light energy absorbed by chlorophyll *b* transfers the energy by a mechanism analogous to resonance to the acceptor molecule (chlorophyll *a*). The underlying principle behind fluorescence measurement is best described by Maxwell and Johnson (2000) "...Light energy absorbed by the chlorophyll molecules in a leaf can undergo one of three fates: it can be used to drive photosynthesis (photochemistry), excess energy can be used dissipated as heat or it can be reemitted as light chlorophyll fluorescence. These three processes occur in competition; such that any increase in the efficiency of one will result in a decrease in yield of the other two…..Hence, by measuring the fluorescence yield, information on the efficiency of photochemistry can be gained." Therefore, the incorporation of energy is inversely proportional to the efficiency of the photochemical complexes and consequently one can use fluorescence measurements to quantify the photosynthetic capacity of a plant, which in turn is inversely proportional to stress (Lichtenthaler, 1995).

Fluorescence has been used to investigate the action of a number of environmental variables on plant conditions, including: water logging (e.g. Percival and Galloway, 1999), salinity (e.g. Belkohogia et al., 1994) and ozone (Grobbelaar and Mohn, 2002). However the mode of measurement should be adjusted to match the experimental context and the species used. Typically fluorescence assessment involves a period of dark pre-treatment then a subsequently saturation of the light harvesting complexes. This may be followed by a subsequently script which repeats the aforementioned saturation although the saturation is done in a backdrop of actinic of coloured light. This exposure to intense bursts of actinic light results in distinct peaks and traces of fluorescence (see figure 5.4). The peak and traces of the fluorescence script correspond to different components of the photoreaction complexes, described in further detail by Carvalho *et al.* (2001) and Zhang and Gao (1999).

The different parameters which relate to the photochemical processes are typically calculated from the baseline fluorescence (Fo) which is the ambient energy emission i.e. without light saturation, the maximum fluorescence (Fm) which results from light saturation and the difference between the two (Fv). A ratio can be produced e.g. Fv/Fm which accommodates species specific difference in actinic light needed for saturation and consequently facilitates cross comparison between species and contexts of stress. Furthermore, the ease of use, versatility of the equipment and ability to compare a number of types of stress has led to fluorescence becoming an increasing popular method to employ in ecophysiological research.

5.3 Objectives

These objectives were to be fulfilled by the following outlined experiments;

- To investigate the mode of stress incurred by leachate irrigation on the foliar metabolism
- To investigate whether cumulative EC is correlated with gas exchange measurements of CO_2 and H_2O flux,
- To compare drought stress with the stress incurred by leachate application.

5.4 Method

5.4.1 Sample population

Figure 5.1: An example of the sample population used for physiological measurements. Note the different colour of the immature leaves.

Leaves from the 6th-12th node from the apex of the dominant branches where chosen for physiological analysis in accordance with Cureton *et al.* (1991), Shrive *et al.* (1990), Ogren (1988), Ogren and Sjostrom (1990), and Ogren and Oquist (1985), this sub population of leaves were chosen for comparability of age and shape and because of the improved exposure to sunlight and reduced influence of predation upon the population.

5.4.2 Gas analysis

The IRGA employed in this study was a CIRAS-1, (PP systems, Hitchin, Herts) coupled with a Parkinson cuvette. Principally it was powered via an external mains connection.

Figure 5.2: The CIRAS-1 on a platform tower set up to take measurements for a PAR curve, note the light source, clamp and variable resistor.

5.4.2.1 Standard protocol

The conditions inside the Parkinson cuvette were determined in separate pilot studies. The following conditions were adopted as standard: leaf area of 3.5 cm a CO_2 concentration of 350 ppm (Ca), a water pressure of 7 mb, a flow rate of 450 $m.mol.s^{-1}$, with an incident actinic light of approximately of > 900 $\mu.mol.m^{-2}.s^{-1}$. Leaves were held horizontally (Linderoth and Cienciala, 1996, Hall *et al.*, 2000) and measurements of gas exchange were conducted on the adaxial leaf surface of these hypostomatous species, after more than one minute but less than five minutes equilibration time, serial measurements were taken.

A constant leaf area was achievable by lining up the margins of the leaf with two marks on the cuvette.

Figure 5.3: A leaf being held horizontally within the cuvette. Note that the leaf does not fill the curette chamber.

Unless stated all measurements were obtained using this protocol.

5.4.2.2 IRGA leaf area

30 leaves were excised from the upper canopy and sliced perpendicular to the vein where the width was 9 mm and then again 45 mm further down the vein. The area of each leaf portion was determined by scanning the portion and analysing the image (using SKYE AEQUITAS image analysis v1.4).

5.4.2.3 *Carboxylation curves*

Four leaves from the two dominant branches were exposed to eight different CO_2 concentrations (from >50-1000 ppm) on various dates throughout the season. The standard IRGA protocol was used except for the varying CO_2 concentrations.

Once steady state conditions were reached serial measurements were taken, dates and cumulative EC are recorded in Table 5.1.

Table 5.1: The EC days of water only and leachate only irrigation treatments and the dates on which CO_2 response curves were performed in the 2002 season.

Treatment	*Date of sample*		
	21/05/02	*26/06/02*	*29/07/02*
Water only	2	5	5
Leachate only	63	167	252

5.4.2.4 *Photosynthetically active radiation response curves*

Using the standard IRGA protocol light response curve conditions were applied to two opposite leaves from three branches for each treatment on the sample dates indicated in Table 5.2

Table 5.2. Dates of PAR curves for the water only and leachate only irrigation treatments in 2002 season with the corresponding EC days.

Treatment	*Date of sample*			
	12/04/02	*16/05/02*	*27/06/02*	*25/07/02*
Water only	1	2	3	5
Leachate only	2	49	167	244

From 9:00-18:00 PAR experiments were performed on leaves selected from the sample population and analysed non-systematically. Serial measurements for each light level were taken once steady state conditions were achieved. Special consideration was given to obtain secure conditions: the branch being sampled was clamped as was the IRGA in order to prevent leaf damage during the experiment. Furthermore the light source was prone to overheating and hence it was decided that a light response curve which progressed from light conditions to dark conditions was more suitable to ensure the longevity of the light source and to circumvent elevated cuvette temperatures.

Irritatingly the PAR sensor for the Parkinson curette was in the way of the light source and therefore had to be removed for the completion of a PAR curve; subsequently the foliar characteristics had to be recalculated retrospectively incorporating the actual incident PAR. Unfortunately the IRGA does not record atmospheric pressure even though it forms the basis of some of the calculations; therefore an ATM of 1000 mb (K Parkinson pers. com) was used.

5.4.2.5 Daytime variation

The standard protocol was used on the sample population, 12 leaves from each treatment were sampled hourly from 9:00-1700 hrs on four occasions through the irrigation period, the dates of sampling and cumulative EC are provided in

Table 5.3 .

Table 5.3: The EC days of the water only and leachate only irrigation treatments and the dates on which diurnal studies were performed in the 2002 season.

Treatment	*Date of sample*			
	14/04/02	*16/05/02*	*18/06/02*	*17/07/02*
Water only	1	2	3	5
Leachate only	3	49	139	218

On a number of dates a non standard protocol was used for gas exchange measurements, where a superambient CO_2 concentration of 700 ppm was used as a reference CO_2 concentration in order to saturate the mesophyll with CO_2. Furthermore measurements commenced whilst the sun was at its zenith.

5.4.3 Water potential of leachate treatment and droughted

Excluding water from the lysimeter planted trees was achieved by covering the surface of the lysimeter with a plastic lining. Special attention was paid to combating the effect of stem flow, drip ropes and ensuring the plastic lining was flush against the stem base remedied the quandary of stem flow to some degree.

Table 5.4: Dates on which equilibrium water potential was measured, special consideration was made to obtain equilibrium water potential measurements within a few days of gas exchange measurements.

Treatment	*Dates of water potential sampling*

Droughted	18/07/01, 26/07/01, 09/08/01, 18/08/01 and 04/09/01.
Leachate irrigation	11/06/01, 06/08/01, 17/07/01, 04/08/01, 21/05/02, 12/06/02, 09/07/02, 03/08/02 and 04/08/02.

The droughted treatment gas exchange was measured during the solar zenith using the standard protocol (outlined in section 5.4.2.1), and the leachate treatment gas analysis data is also from the solar zenith using the daytime studies.

5.4.4 Chlorophyll fluorescence script and protocol

Leaves from the sample population of the trees, not used for gas analysis, were non-systematically selected for chlorophyll fluorescence measurement. Separate pilot studies were carried out in order to determine a useable script. These pilot studies were repeated on six separate samples. A script was determined by first measuring the fluorescence of a dark adapted leaf. Adjustment of the distance between the fibre optic cable and the sample ensured a base signal (Fo) is within range of 100-700 bits. Once this was achieved the distance between the fibre optic cable and the sample was kept constant using rubber spacers. Determining a sufficient saturating pulse to induce maximum fluorescence (Fm) was achieved by subjecting replicate samples to increasing saturation pulses from 1800 to 18,000 μmol.m^{-2}.s^{-1} at increments of 1800 μmol.m^{-2}.s^{-1}. The minimum saturation pulse which induces a comparable Fm was used to reduce the likelihood of damage to the photosystems from over exposure. The time used to measure Fo and Fm was set in accordance to the manufacturers' settings.

Consequently, scripts employed a time lag of 11 seconds to measure Fo and a saturation pulse of 12600 µmol.m^{-2}.s^{-1} for 0.7 seconds was employed.

Each leaf was dark adapted for at least 30 minutes using a 7 mm leaf clip, excised and then subjected to the prepared script using the FMS II (Hansatech instruments, Norfolk, UK). Chlorophyll fluorescence measurements were conducted on nine leaves per treatment, between 9:00-19:00, at two hour intervals, on four dates (see Table 5.5 for dates and EC days of sampling).

Table 5.5: Dates and EC days (DS.m^{-1}.day) of diurnal chlorophyll fluorescence measurements for water only, cyclical water:leachate and leachate only irrigation treatments.

Treatment	*Date of sample*			
	11/04/02	*19/05/02*	*21/06/02*	*27/07/02*
Water only	1	2	3	5
Cyclical leachate:water	1	45	80	131
Leachate only	2	63	151	252

5.5 Results

5.5.1 Gas analysis

5.5.1.1 Carboxylation curves

Figure 5.4 demonstrates interesting variation but a consistently a positive relationship between calculated CO_2 uptake and sub stomatal CO_2 concentration. The water only irrigation treatment is illustrated as an average throughout the season as there are non significant differences between the dates of sampling (see **Table** 9.2). The plateau regions on Figure 5.4 shows that there is a reduction in RuBP/ RuBISCO turnover with persistent leachate irrigation.

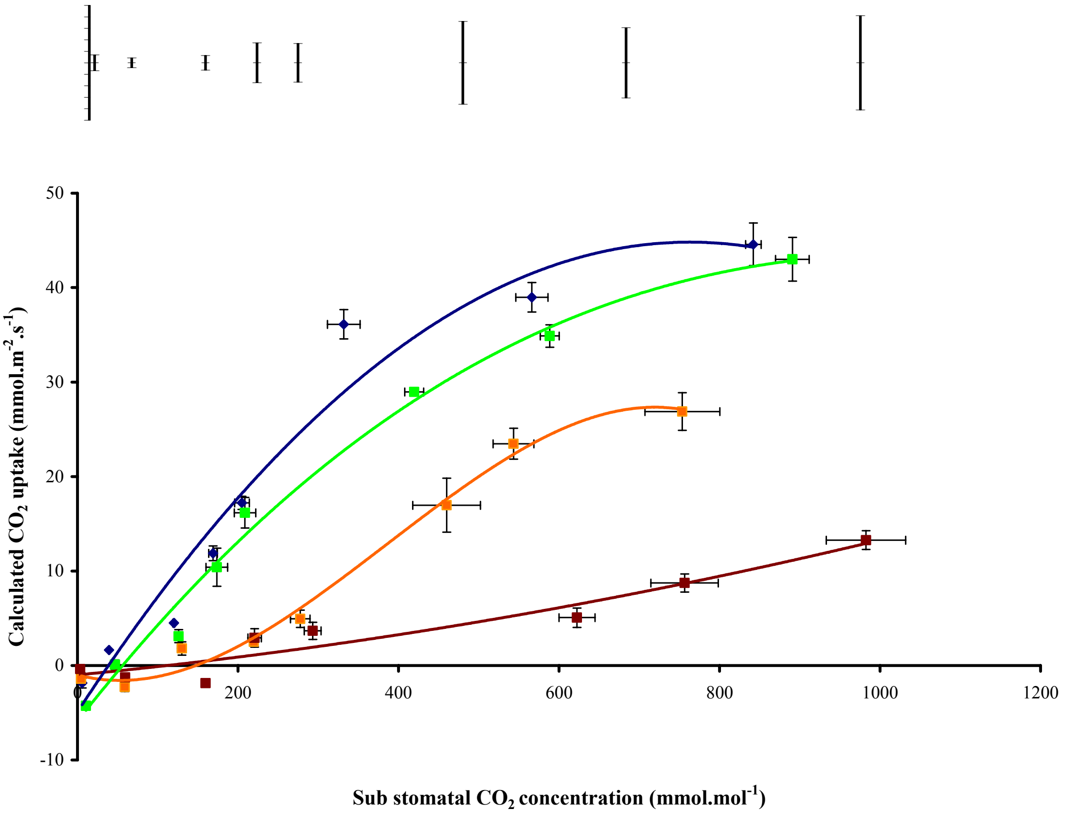

Figure 5.4: Carboxylation curves for water only irrigation treatment, here presented as the mean of the three carboxylation curves obtained (blue), leachate only treatment on 21/05/02 (green), on 26/06/02 (orange) and 29/07/02 (red). Error bars are standard error of the mean and the bars above the plot area are LSDs.

Furthermore from the differences in the initial linear phase of the carboxylation curve a reduction in carboxylation efficiency is apparent, Table 5.6 illustrates this more clearly showing an 80% decrease after a cumulative EC of 167 $DS.m^{-1}$.day, and then subsequently a 90% decreases after 252 $DS.m^{-1}$.day.

Table 5.6: Showing the Km for RuBISCO as determined from the zero to 350 ppm Ca for the persistent leachate and water only irrigation treatments over the 2002 experimental season (EC days is in brackets).

Treatment	*21-May*	*26-Jun*	*29-Jul*
Water only	0.09 (2)	0.12 (3)	0.08 (5)
Leachate only	0.10 (63)	0.02 (167)	0.01 (252)

The shape of the leachate only irrigation treatment carboxylation curves is similar to that of the average water only irrigation treatment (average EC days 3 DS.m^{-1}.day) despite an increase in EC days (to 63 DS.m^{-1}.day and 167 DS.m^{-1}.day) although interestingly the A/Ci curve obtained on 252 DS.m^{-1}.day shows a more linear rather than a sigmoidal shape.

5.5.1.2 PAR response curves

Figure 5.5 shows that with persistent application of leachate the maximum CO_2 uptake is inhibited at saturating irradiances. The water only irrigation treatment is illustrated as an average throughout the season as there are non significant differences between the dates of sampling (see Table 9.3).

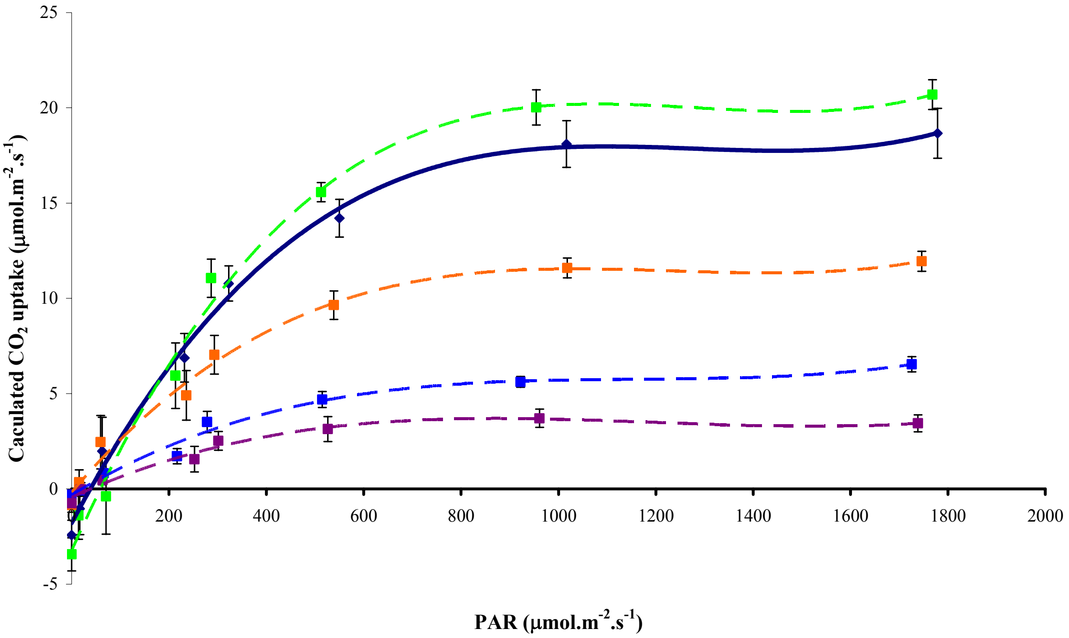

Figure 5.5: Light response curves here presented as the mean of the four carboxylation curves obtained for the water only irrigation treatment (royal blue), leachate only treatment on 12/04/02 (green), on 16/05/02 (orange), 27/06/02 (light blue) and 25/07/03 (purple). Error bars are standard error of the mean and the bars above the plot are LSD's.

Statistical determination of LSD reveals that at low irradiances < 300 µmol.m^{-2}.s^{-1} there is no significant difference between the water only and the leachate only irrigation treatment at any point during the experimental period, however light response curves obtained from an EC day of 49 DS.m^{-1}.day onwards are all significantly different ($p < 0.001$) from the water only irrigation treatment at high irradiances (> 500 µmol.m^{-2}.s^{-1}, see Table 9.2). Also the initial light response curve for leachate only irrigation treatment reveals a slight difference in maximum calculated CO_2 uptake relative to the water only irrigation treatment, although the LSD bars in Figure 5.5 illustrate that the differences were not significant. Furthermore the magnitude of difference between the leachate only and the water only irrigation treatments get progressively larger as leachate irrigation persists.

Finally all light response curves show that an irradiance of approximately 800 – 1000 µmol.m^{-2}.s^{-1} is sufficient to saturate the photosynthetic complexes to a degree where PAR is not a limiting factor for the uptake of CO_2 as indicated by the plateau in carboxylation.

5.5.1.3 Gas analysis over the experimental period

At the beginning of the 2001 season there were no statistically significant differences between the leachate only and the water only irrigation treatment. However after 63 days of persistent leachate irrigation, which corresponded to an EC days of 197 DS.m^{-1}.day the calculated CO_2 uptake, stomatal conductance and transpiration are all reduced significantly ($p = 0.001$) and sub stomatal CO_2 concentration is increased significantly ($p = 0.001$) relative to the water controls. The scale of these differences was a 39% suppression of calculated CO_2 uptake, a 33% decrease in stomatal conductance and a 17% reduction in transpiration. The magnitude of these relative decreases was somewhat less after an EC days of 224 DS.m^{-1}.day (obtained on the 73rd day of leachate irrigation). When the suppression of calculated CO_2 uptake, stomatal conductance and transpiration was 17, 21 and 9% respectively. Furthermore the statistically significant ($p > 0.001$) increase in sub stomatal CO_2 concentration after 197 DS.m^{-1}.day isn't replicated after 224 DS.m^{-1}.day where no statistically significant differences ($p = 0.957$) were recorded.

Table 5.7: Average foliar parameters over the course of a daytime study, where A is calculated photosynthetic rate (μmol.m^{-2}.s^{-1}) Gs is stomatal conductance (mmol.m^{-2}.s^{-1}) E is transpiration (mmol.m^{-2}.s^{-1}) and Ci is Sub stomatal CO_2 concentration (μmol.mol^{-1}), on 25/05/01, 29/07/01 and 14/08/01.

Treatment	**Days from irrigation commencement**											
	0				**63**				**73**			
	A	*Gs*	*E*	*Ci*	*A*	*Gs*	*E*	*Ci*	*A*	*Gs*	*E*	*Ci*
Leachate only	19.9	217.1	3.3	151.4	9.1	126.3	2.9	191.5	11.0	101.4	2.3	139.4
Water only	19.3	256.7	3.5	165.3	14.9	188.0	3.5	158.7	13.8	135.9	2.5	139.8
LSD	0.8	36.2	0.3	23.3	1.0	28.1	0.3	18.2	1.0	16.2	0.3	14.2
p =	0.393	0.032	0.181	0.239	0.001	0.001	0.001	0.001	0.001	0.001	0.11	0.957

Unlike 2001 at the beginning of the 2002 season there were statistically significant differences between water only and the leachate only irrigation treatment. Unlike the final dataset from the 2001 season the leachate only treatment had increased average daytime stomatal conductance, transpiration and sub stomatal CO_2 concentrations (by approximately 14 %). Similar to the 2001 season the leachate only treatment gas exchange was suppressed with leachate irrigation. After an EC days of 145 $DS.m^{-1}$.day calculated CO_2 uptake, stomatal conductance and transpiration were decreased by 27, 35 and 26% respectively relative to the water only treatment on the day of sampling. After an EC days of 218 $DS.m^{-1}$.day the largest significant difference between averages is apparent where a suppression of calculated CO_2 uptake, stomatal conductance and transpiration (73, 55 and 44% respectively) and an highly significant ($p < 0.001$) increase in sub stomatal CO_2 concentration (26 %), whereas in 2001 an EC days of 224 $DS.m^{-1}$.day was achieved yet only significant differences between stomatal conductance and calculated CO_2 uptake were recorded.

Table 5.8: Average foliar parameters over the course of a diurnal study, where A is calculated photosynthetic rate (μmol.m^{-2}.s^{-1}) Gs is stomatal conductance (mmol.m^{-2}.s^{-1}) E is transpiration (mmol.m^{-2}.s^{-1}) and Ci is sub stomatal CO$_2$ concentration (μmol.mol^{-1}), on a number of dates in the 2002 experimental period.

Treatment	***Days from irrigation commencement***															
	0				**30**				**65**				**91**			
	A	Gs	E	Ci	A	Gs	E	Ci	A	Gs	E	Ci	A	Gs	E	Ci
Leachate only	14.4	243.2	3.2	220.7	13.4	155.6	2.6	170.7	10.5	207.0	2.9	233.0	5.1	136.0	2.5	250.4
Water only	15.6	214.3	2.8	194.1	18.3	240.4	3.5	177.7	16.8	292.3	3.6	212.9	18.9	301.9	4.4	198.4
LSD	1.1	20.2	0.2	9.1	1.0	16.2	0.2	11.5	1.0	24.0	0.2	9.3	0.8	19.3	0.2	11.4
p =	0.039	0.005	0.004	0.001	0.001	0.001	0.001	0.23	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001

The change in calculated CO_2 uptake follows a linear reduction (see Figure 5.6) in both years (where $R^2 = 0.857$ in 2002 and $R^2 = 0.57$ in 2001).

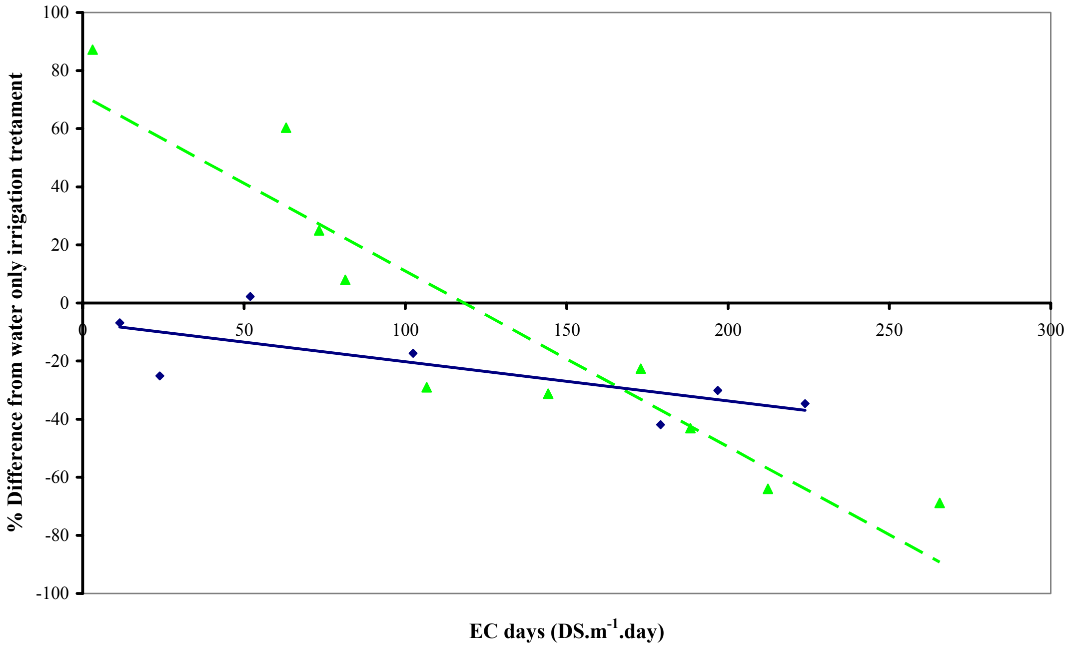

Figure 5.6: Changes in parameters associated with carboxylation expressed as a percentage of the water only irrigation treatment, on a number of days through the 2002 season. The dashed green series is calculated CO_2 uptake in 2002 and the solid blue series is the calculated CO_2 uptake in 2001.

Figure 5.6 shows that suppression in factors associated with carboxylation follows a linear trend for *Salix viminalis* under leachate irrigation. Also Figure 5.6 demonstrates that the rate of suppression in calculated CO_2 uptake in 2001 was less severe than in 2002. Furthermore it is evident that in 2002 the continuous leachate irrigation treatment had, initially, a higher calculated CO_2 uptake (80% more) which progressed to a relative reduction (70%) after leachate irrigation corresponding to 250 DS m^{-1}.day.

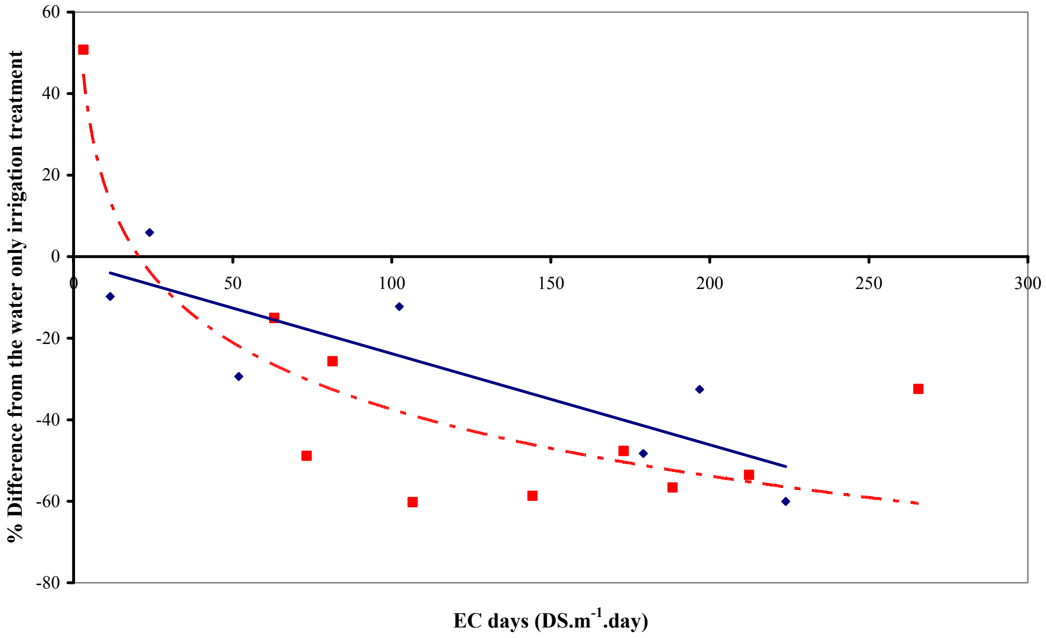

Figure 5.7: Changes in stomatal conductance (Gs) under leachate irrigation the data is expressed as a percentage of the water only irrigation treatment, obtained on a number of days through both irrigation periods. The 2001 experimental period is denoted by the blue solid line and the 2002 experimental period is denoted by the red dashed line.

Stomatal conductance unlike the trend demonstrated by the calculated CO_2 uptake demonstrates a different trend between the two years, where a linear trend fits the 2001 data best ($R^2 = 0.72$) and an exponential fitted line is more appropriate for the 2002 data ($R^2 = 0.84$). Like calculated CO_2 uptake in the 2002 season, stomatal conductance is initially higher than the water only irrigation treatment (+ 50%) but leachate irrigation instigates a rapid reduction in stomatal conductance.

The overall trend of gas flux is comparable between studies using ambient and superambient reference CO_2 conditions even for transpiration and sub stomatal CO_2 (data not shown). Furthermore the magnitudes of differences are very similar, with the exception of the initial measurements. Superambient CO_2 conditions helped to illuminate a treatment difference before the season's irrigation schedule has commenced: the leachate irrigation treatment had a higher calculated CO_2 uptake and stomatal conductance.

5.5.2 Water potential of leachate treated and droughted willows

A strong linear decrease in calculated CO_2 uptake was apparent for both the droughted ($R^2 = 0.84$) and leachate irrigated trees ($R^2 = 0.87$).

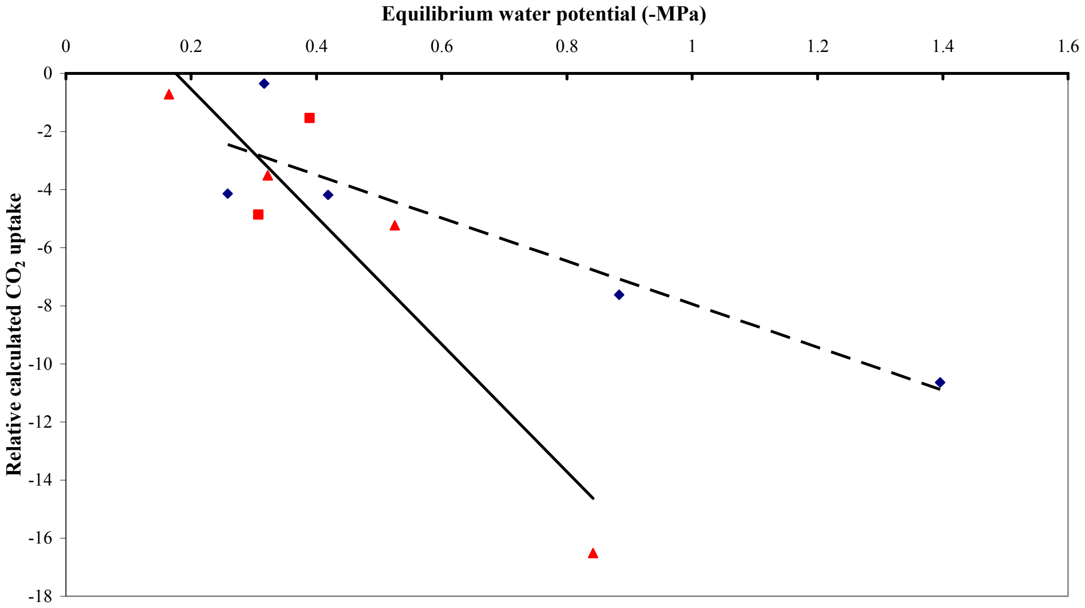

Figure 5.8: Water potential and relative calculated CO_2 uptake of a droughted (dashed line), and persistent leachate treatment (solid line) relative values are determined by the closest control measurement.

However there is a difference between the droughted and leachate treatment, which shows that the leachate treatment experiences an accentuated rate in decline of calculated CO_2 uptake relative to the water only treatment when compared to the droughted treatment.

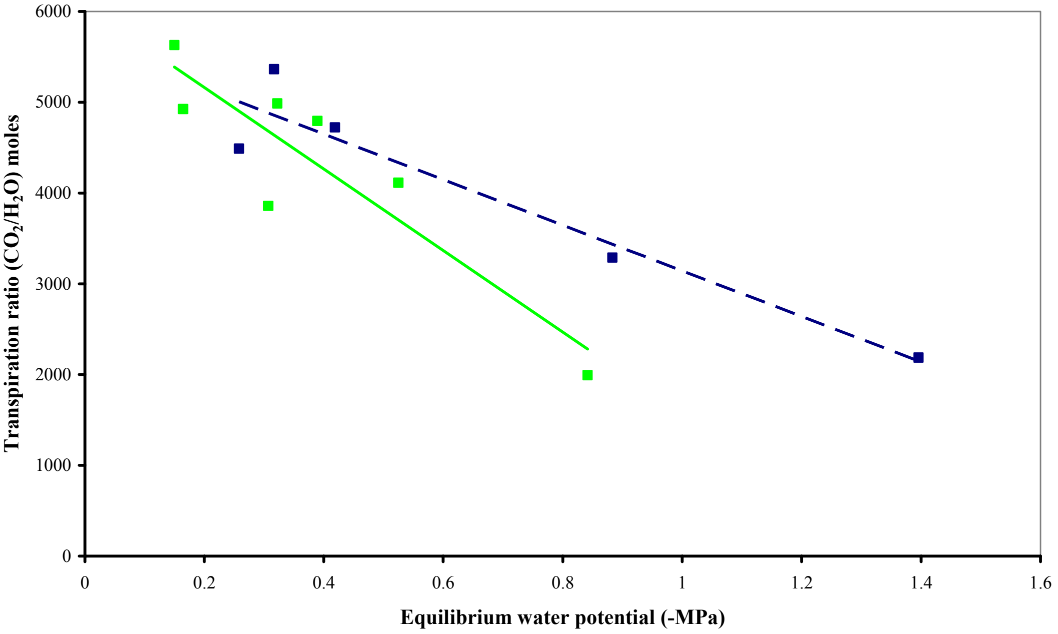

Figure 5.9 Water potential and transpiration ratio of a droughted (blue dashed line), and the persistent leachate treatment in 2001 and 2002 (green solid line).

Figure 5.9 demonstrates a similar response for transpiration ratio to that for CO_2 uptake. Again a linear trend was observed (R^2 = 0.912 for the droughted and R^2 = 0.8199 for the leachate only irrigation treatment) and again the rate of decrease is greater for the leachate only treatment. In both Figure 5.8 and Figure 5:11 a zero value is never reached, leaves are excised before a stasis in gas flux was measured.

5.5.3 Chlorophyll fluorescence

Figure 5.10 shows that there is very little difference in Fv/Fm between treatments at the start of the experimental season.

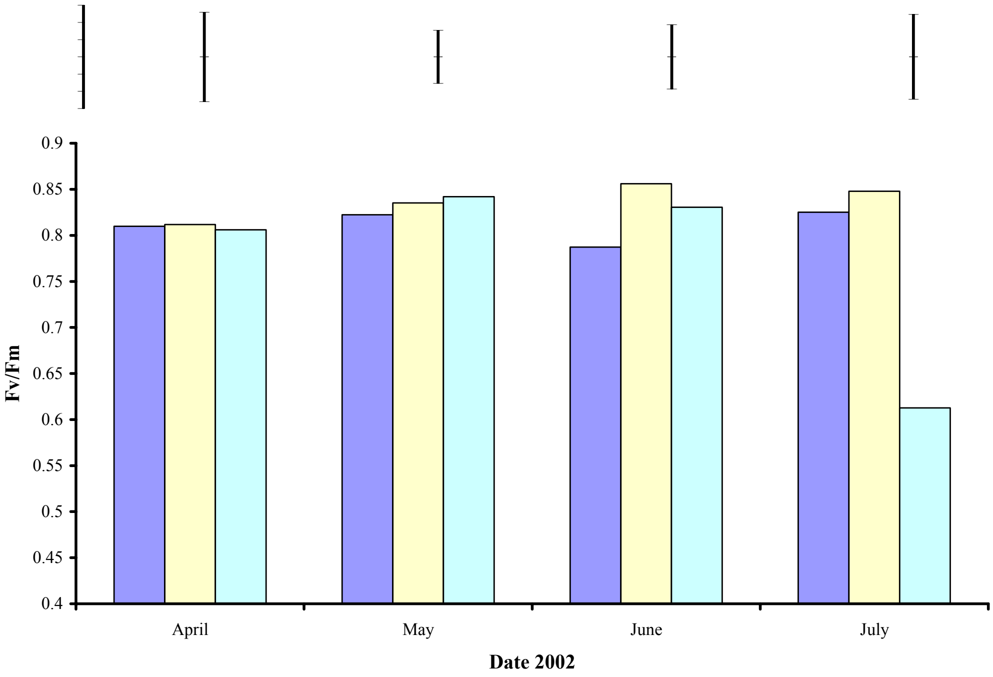

Figure 5.10. Dark adapted fluorescence measurement of Fv/Fm for the water only (blue), cyclical leachate:water (yellow) and leachate only irrigation treatments (green), taken on 11/4, 19/05, 21/06 and 27/07 in the 2002 experimental period . Above the bar chart are the calculated least significant differences, grouped by date of sample.

The leachate only irrigation treatment is only significantly different from the water only and cyclical water:leachate irrigation treatments when sampled in July. The cyclical water:leachate irrigation treatment does not exhibit a significantly different Fv/Fm ratio at any date of sample, despite reaching a cumulative EC of 131 DS.m^{-1}.day. However there are significant differences ($p = 0.03$) between data obtained for all treatments in June and July compared with data taken earlier in the year.

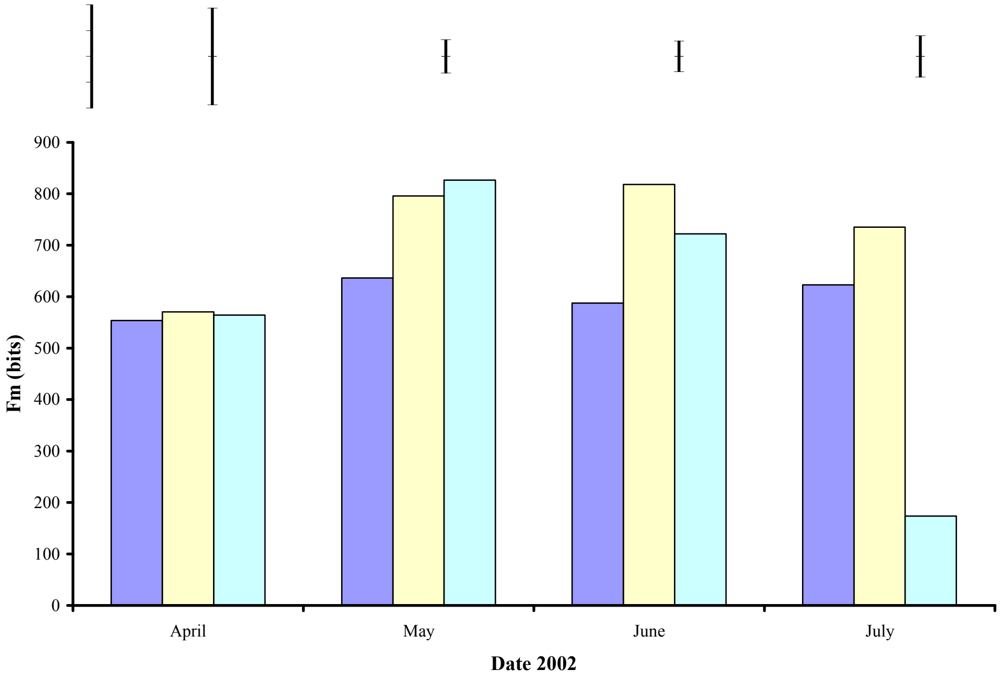

Figure 5.11: Dark adapted fluorescence measurement Fm for the water only (blue), cyclical leachate:water (yellow) and leachate only irrigation treatments (green), taken on 11/4, 19/05, 21/06 and 27/07 in the 2002 experimental period. Above the bar chart are the calculated least significant differences, grouped by date of sample.

Figure 5.11 shows that the Fm values for the water only treatment do not change significantly over the season. The Fm values for the cyclical water:leachate irrigation treatment and the leachate only irrigation treatment are significantly ($p < 0.001$) higher than the water irrigation treatment by the May measurement. By June the leachate only treatment is significantly lower than the cyclical water:leachate treatment when EC days were 151 DS.m^{-1}.day, and 80 DS.m^{-1}.day respectively. Moreover the leachate only treatment is significantly lower than the cyclical water:leachate and the water only irrigation treatments by July.

Slow phase characteristics are not shown because only 4 days were sampled and contained anomalous data for one of those days.

5.6 Discussion

Irrigation with domestic landfill leachate supplies high loads of nitrogen and chloride. Therefore a plant irrigated with leachate is liable to stress from loading both excesses. Physiological responses to nitrogen status and salinity stress have been investigated thoroughly, although interactions between the two have been less well investigated, with notable exceptions, e.g. Shagguan *et al.* (2000).

High Fm values (Figure 5.11) and relatively high initial gas exchange (Figure 5.6) indicates that the leachate only irrigation treatment is nutrient enriched relative to the water only irrigation treatment. The elevated initial foliar gas exchange recorded in Figure 5.6 could be a result of increased concentrations of RuBISCO which has been proven to correspond to leaf nutrient status (Raven and Sprent, 1993 and Evans, 1983). Furthermore Evans (1983) and Shangguan *et al.* (2000) found nitrogen nutrition resulted in elevated gas exchange. However a relationship between nitrogen nutrition and a stimulation of parameters associated with water vaporization is less obvious. A feed forward response attributable to elevated carboxylation is one possible explanation, but also an increase in individual leaf area could also explain the relatively high stomatal conductance observed in this experiment.

Plant nutrient status also influences the physiological response to drought. Foliar nitrogen status has been shown to accentuate the stress response in some species e.g. beans, (Shimishi, 1970) and winter wheat, (Shangguan, 1997). Predominantly however, nitrogen status has been shown to increase resistance to water stress (Shangguan *et al.*, 2000, Evans, 1983, Latiri-Souki *et al.*, 1998 and Ogren, 1988a). Although cross comparison is difficult because of the different experimental aims and designs, it is likely that the response is highly species specific. It is noteworthy that Ogren (1988) found *Salix* sp. (not specified which species) to be highly drought sensitive under sub optimal nitrogen status. Subsequent papers have added clarity to the mechanism by which nitrogen status could alleviate water stress in riparian species (Lowenstein and Pallardy, 1998). Consequently if one was to compare a relatively nitrogen enriched *Salix* population under water stress conditions one would expect some degree of attenuation of the stress response in the nutrient enriched treatment. However in Figure 5.9 it is apparent that leachate irrigation accentuates the stress response, in this case transpiration ratio. Takacs *et al.* (2001) found an exaggerated stress response of *Tortula ruralis* in the presence of a toxic solute, although *Tortula ruralis* is a Bryophyte and therefore genotypically highly dissimilar to *Salix* sp. Adams *et al.* (1992), Pinheiro *et al.* (2001) and Tattini *et al.* (2002) have found that significant osmotic adjustment with increasing salt concentrations. An accumulation of chloride in the leaves could be a drain on chemical energy due for example to a portioning of energy for osmotic adjustment which may result in an exaggeration of the stress response in the leachate only irrigation treatment.

However the differential response between the leachate and droughted treatment could be attributed to the measurement of equilibrium water potential. The solute potential was never recorded, which considering the possibility of osmotic adjustment is worthy of study. Also the equilibrium water potential may not be a fair indication of the soil water status (Sellin, 1999). Equilibrium water potential is reliant on a cessation of gaseous flux which results in the plant water equilibrating with the soil water potential, however night-time transpiration has been recorded and it is thought to result in elevated water potentials (Donovan *et al.,* 2001). Arguably if the leaves were analyzed for water potential on the same day they would be exposed to the same conditions, but the droughted treatment was analysed on different days from the leachate only and the water only irrigation treatments due to temporal restrictions. Furthermore, Sellin (1999) reported that equilibrium water potential may occur a few hours after dawn due to the magnitude of biomass and distance water has to travel in tree species. This was unlikely to be a problem because of the choice of sample population for equilibrium water potential analysis and the size of the coppiced plants.

In addition to the differential trends exhibited by the persistent leachate and droughted treatments it is noteworthy that both the persistent leachate and droughted leaves never achieved a stasis in gas exchange, although it is possible that gas exchange did reach zero before leaf drop. However other researchers have found that leaves may persist despite gas stasis (W Stephens, pers. com. 2002, L Bonneau pers. com. 2002, Lebourgeois *et al.,* 1998). Associated research has found that hybrids of *Salix viminalis* and *S. nigra* are prone to premature excision (L. Bonneau, pers com. 2002, Macleod *et al.,* 1986, Pitcher and McKnight, 1990 cited in Lowenstein and Pallardy, 1998 and Ogren, 1988). Leaf excision before gas exchange stasis is achieved is indicative of a species with a high energy quotient, i.e. if carboxylation is reduced beyond a threshold, which dictates whether the leaves are productive the leaves are sacrificed. Therefore, further investigation is recommended to elucidate the threshold of gas exchange necessary for leaf persistence. Experimental evidence in chapter 4 showed that periodic flushing with water resulted in leaf persistence. Possibly a recovery of gas exchange could occur, but this requires further investigation. A supplementary finding from the droughted/leachate comparison is that the equilibrium water potential of the leachate only treatment did not fall below –

0.8 MPa (the droughted reached –1.4 MPa). This indicated that factors other than water status were responsible for leaf abscission. Given the assertion that *Salix viminalis* leaves have a high energy quotient for their retention; one can deduce that the reduction in carboxylation and subsequent leaf abscission can not be attributed solely to an increase in soil matric potential.

A suppression of gas exchange in response to salinity has been recorded by a number of authors (Delfine, *et al.*, 1999, Tattini, *et al.*, 2002, Sifola and Postiglione, 2002, Loreto, *et al.,* 2003). Moreover suppression in gas exchange has been recorded with leachate irrigation (Cureton *et al.,* 1991, Shrive *et al.,* 1990). In this study a suppression of gas exchange has also been recorded. However comparison between the three studies at a specific point in time is difficult because of the way each study has presented the irrigation schedules, the different leachates used and the different plant materials employed. However Cureton *et al*. (1990) recorded an increased suppression of gas exchange at the end of the second year of leachate irrigation. Using Table 5.7 and Table 5.8 one can see that the suppression of gas exchange is highly comparable between diurnal studies performed on the 63rd and 65th day of leachate irrigation, in 2001 and 2002 respectively. Calculated CO_2 uptake, transpiration and stomatal conductance were all reduced by 39, 33 and 17% respectively in 2001 and in 2002 calculated CO_2 uptake, transpiration and stomatal conductance were suppressed by 38, 29 and 19 % respectively in 2002. However the similarity in gas exchange suppression is not reciprocated if one uses EC days as a predictor, and therefore may highlight some of the shortcomings of EC days. It is possible that the different ages of the plant material and prior treatment is a factor in the different response found in this study and further experimentation may improve the use of a cumulative EC indicator. Nevertheless the adoption of EC days will facilitate cross comparison between future investigations. Due to the lack of information given in Cureton *et al.* (1991) and other studies focused on leachate irrigation cross comparison thus far is impossible.

Unlike Shrive *et al.* (1990) and Cureton *et al.* (1991) fluorescence techniques were employed in order to monitor light acquisition. From Figure 5.10 it is apparent that the capture of light is relatively unaffected by leachate irrigation until an EC days of 252 DS.m^{-1}.day. By this stage the leaves were unsuitable for gas analysis due to prolific curling and encroaching necrosis. Carboxylation on the other hand demonstrates a significant ($p = 0.01$) suppression after an EC days of 143 and 197 DS.m^{-1}.day and in 2002 and 2001 continues to be significantly decreased with persistent leachate irrigation relative to the water only treatment. Therefore one can deduce that the dark reactions are more affected by the leachate irrigation than the light reactions. This finding concurs with previous work done by Ogren and Oquist (1985) who conclude that carboxylation is most affected by drought. Furthermore from Table 5.6 and Figure 5.4 it is apparent that a significant ($p = 0.01$) impairment of both the Km of RuBISCO and RuBP/RuBISCO regeneration capacity. Again this concurs with Ogren and Oquist (1985) who concluded that the decrease in carboxylation was mainly due to non stomatal factors i.e. RuBISCO activity, and therefore RuBISCO is the most drought sensitive component of the photoreaction complexes. Therefore it is possible that the leachate only irrigation treatment adopted the same stress response to leachate as it would to drought.

Salt accumulation and osmotic inhibition (Adams *et al.,* 1992, Durr, 1996, Dowlen, 1997 and Choisel, 1997) may not be the only causal agents of the stress response. In Section 6 the possibility of water-logging was discussed. The leachate only treatment could potentially be exposed to water logging, salt and osmotic stress, which in combination could compound the stress response and explain the exaggerated trend. Ultimately leachate stress is comparable to water stress, because all the components result in a restriction of available water for metabolism albeit with a number of additional restrictive factors. For example Kaiser *et al.* (1981) found that osmotic cellular stress acts primarily upon the dark reactions and ATP synthesis rather than on the primary photoreactions and electron transport. Furthermore Delfine *et al.* (1999) found that salt accumulation impairs RuBISCO activity. This therefore may explain the accentuated rate of stress response exhibited by the leachate only irrigation treatment.

Despite showing that carboxylation was most affected by drought stress, Ogren's subsequent papers concerning *Salix* species succumbed to fashion and employed fluorescence techniques using these surrogates to quantify stress in *Salix* species (Ogren, 1988 and Ogren and Sjostrom, 1990). Numerous authors have reported the phenomenon of photoinhibition e.g. Bjorkman and Powles, (1984), Bjorkman *et al.*, (1988) and Ogren, (1988b). Moreover Ogren and Sjostrom, (1990) found that *Salix* species in field conditions suffered photoinhibiton under droughted conditions. Photoinhibiton was not apparent in the leachate only irrigation treatment (data not shown). The prospect of photoinhibiton influenced the way the data was obtained and presented significantly; averaged gas exchange parameters were presented in order to accommodate photoinhibition or unusual diurnal trends.

A number of authors e.g. Zhang *et al.* (2001), Blackman and Davies (1985), Cornic and Miginiac (1983) and Tillberg *et al.* (1981) have found a strong relationship between foliar ABA concentrations and drought. Accordingly Lowenstein and Pallardy (1998) found a strong relationship between biophysical/chemical signals and stomatal response for a number of tree species. Most fine roots occur in the topsoil (Lebourgeois *et al.,* 1998) and nitrogen application in the early stages of growth increase fine root proliferation (Aronsson and Bergstrom, 2001) moreover the top soil was less compacted and combined with Biogran^{TM} therefore providing more favourable conditions for root (especially fine root) proliferation. EC was measured at a depth of 0.1 m, the close association of the fine roots and measured EC coupled with the strong chance that the biophysical/chemical signal which controls stomatal aperture emanates from these roots makes it unsurprising to find a strong ($R^2 = 0.84$ in 2002) between EC days and stomatal conductance. The stomatal response follows a rapid exponential decline, which is indicative of a plant with access to a low volume of exploitable soil under stress (FAO, 1998).

5.7 Conclusions

The study of the physiological response of *S. viminalis* to landfill leachate irrigation indicated that the gas exchange of *S. viminalis* is significantly suppressed by leachate irrigation. Furthermore relative to a droughted treatment the gas exchange of *S. viminalis* is suppressed at an accentuated rate. Conversely energy transference is relatively unaffected by leachate irrigation, until chronic foliar conditions occur.

6 Soil conditions inside the Lysimeter

6.1 Introduction

An epinastic response was observed in some treatments prior to defoliation which suggested a reaction to unfavourable soil physio-chemical conditions. Destructive sampling enabled investigation of soil physio-chemical properties.

6.2 Literature review of soil conditions resulting from leachate irrigation

6.2.1 Soil chemistry

There have been a number of studies which have concluded or at least alluded to salinity being the primary constraint to growth under leachate irrigation (Cureton *et al.,* 1991, Wong and Leung 1989, Durr, 1996, Choisel, 1997, Dowlen 1997, Stephens *et al.,* 2001, Tieberghein, 1998). Despite the widespread agreement about the restrictive effect of salinity upon a landfill leachate attenuation system no studies have investigated the individual components of salinity or their distribution with different management regimes. Salinity has been demonstrated to have a significant effect on consumptive water use (Basil and Kaffka, 2002) yield (De Pascale and Barbieri, 1997) and primary metabolism (Steduto *et al.,* 2000). Water extraction by plants may be diminished under saline conditions which may have consequences on soil physio-chemical properties.

6.2.2 Soil physio-chemistry

Numerous studies have demonstrated the importance of oxygen diffusion rate (ODR) and redox on plant welfare (Warnaars *et al.*, 1970, Phene *et al.*, 1976, Stolzy and Letey, 1964). But the effect of leachate irrigation on these properties and their impact on coppiced *Salix viminalis* has not been investigated.

6.2.2.1 Redox potential

The tendency of a solution to donate electrons to a reducible substance or to accept electrons from an oxidisable substance is called "redox potential", (Hillel, 1998). Redox potential (Eh) is a measure of the free energy in a solution and is expressed using Joules, Volts or Calories

When an inert electrode is placed in a solution the movement of electrons to the electrode surface is analogous to "pressure" insofar as the electro-potential of the solution is proportional to the concentration of electrons. In soils the electrons emerge from geological and biological sources.

Redox potential is commonly used as an indicator of the potential and concentrations of oxidised and reduced compounds in the soil and therefore energy levels of the soil. From

Table 6.1 one can see that oxygen is reduced at 330mV, which is high relative to the others on the table. Subsequently redox potential is commonly used to investigate the aeration status of a soil (Dasberg and Bakker, 1970). The reduction in availability of oxygen in the soil is usually attributed to the exclusion of oxygen by the invasion of water into otherwise air filled pockets. The subsequent exclusion of oxygen initiates its reduction and release of energy. Redox potential is usually measured potentiometrically using an inert platinum anode connected to a high resistance voltmeter and a reference hydrogen electrode.

Table 6.1: The relationship between energy status of the soil and the effect on different reducible or oxidisable components in the soil (Gambrell and Patrick, 1978).

Component	*Eh mV*
Disappearance of O_2	+330
Disappearance of NO^{3-}	+220
Appearance of Mn_2	+200
Appearance of Fe_2	+120
Disappearance of SO^{2-}	-150
Appearance of CH_4	-250

The reference cell electrolyte solution to seeps through the ceramic junction to make the necessary electrical connection. This reference cell provides a small voltage which, relative to the voltage of the Pt electrode, is an indication of electron intensity. Therefore the voltage difference between the reference electrode and the inert anode is the measure of the reactive species.

Initially a hydrogen cell was used for the reference electrode however for ease commonly a silver-silver nitrate with a potassium chloride electrolyte solution or a calomel reference electrode are used instead. These electrodes have different electrochemical properties when in contact with a soil, so one needs to convert the measured E to Eh by accounting for the reduction in potential generated by the electrode used (relative to a hydrogen electrode). The silver-silver nitrate and calomel electrodes produce a smaller voltage than the hydrogen method therefore Rowell (1988) states that if one uses either of these electrode one must convert the measured E in accordance to the reference electrode in order to generate useable measurements:

$Eh = Eh_m + 248$ mV (Calomel)

$Eh = Eh_m + 207$ mV (Ag-Ag nitrate with KCl)

Equation 6.1: Equations used for the conversion of Ehm to Eh at temperatures of 25°C for the two main types of reference probes (from Rowell, 1998), where Eh_M = the measured Eh (mV).

Special consideration needs to be made to ensure fully reduced conditions on the platinum electrode. For example, if the electrode is partially oxidised one can expect lower redox readings. Therefore some authors recommend one should bubble hydrogen over the electrode e.g. Hillel (1998). Bubbling hydrogen over an electrode between readings is not practical and so alternatively one can insert both electrodes into standard redox solution and measure the potential to ensure the platinum electrode is reduced (Rowell, 1988). Rowell (1981) offers a further option of cleaning the electrode with acid between samples.

Numerous secondary factors influence Redox potential, each unit of pH (Hillel, 1998 & Rowell, 1988) is assumed to instigate a 59 mV change in Eh. Therefore for comparable results Glinski and Stepniewski (1985) and others encourage Eh to be normalised to pH 7, using Equation 8.

$Eh_7 = Eh_M - 0.059\ (7 - pH)$

Equation 6.2: For normalisation of Eh to pH, where; Eh_7 = the Eh normalised to pH 7 and Eh_M = is the measured Eh (mV) (Glinski and Stepniewski, 1985).

Furthermore, temperature influences the instantaneous reading of Eh, through altering the viscosity of water, diffusion of oxygen and the resistance of the equipment, additionally increased temperature affects the metabolism of micro-organisms which contribute to the disassociation of the free energy. Soil chemical factors have an impact on Eh; reducible organic matter, and inorganic compounds such as nitrates (because they are electron acceptors) influence microbial activity and subsequently redox potential.

6.2.2.2 Oxygen diffusion rate

Oxygen diffusion rate (ODR), typically measured in $g.cm^{-2}.min^{-1}$ is the assumed diffusion rate of oxygen to a root tip in water. ODR is largely used as a surrogate for the aeration status of a soil. Its importance has been reviewed by Stolzy *et al.* (1975). Plants require an adequate supply of oxygen in order to facilitate maximum absorption of minerals. Prolonged soil oxygen deficiency causes an increase in cell wall permeability and ultimately root death (Williamson and Kriz, 1970 and Campbell and Seaborn, 1972).

Early methods to assess the aeration of soil conditions have involved the extraction of gas samples and subsequent analysis of oxygen and carbon dioxide or have used an oxygen deficient absorber buried in soil (Tackett, 1968). However once the diffusion rates of oxygen through water was determined (approximately 10000 times lower than atmospheric diffusion) a more realistic approximation of the oxygen supply to respiring roots were made possible using electrochemical methods (Lemon and Erickson, 1952, Letey and Stolzy, 1962). The early methods involved measuring the current using a high resistance ammeter across an inert platinum (Pt) cathode with a regulated applied voltage (usually using a variable resistor and a DC power supply, see Figure 6.3), and a calomel reference electrode (silver-silver nitrate reference cell with saturated potassium chloride electrolyte).

The theoretical basis of the polargraphic ODR measurement relies on the diffusive gradient instigated by a respiring root. A healthy respiring root constantly depletes oxygen levels in the surrounding water films and therefore maintains an oxygen gradient which results in the diffusion of oxygen from air pockets to the soil water. The soil-water diffusion step is the limiting factor in the process. Therefore by measuring the oxygen diffusion rate from soil pores to water, one can approximate the potential oxygen supply to roots. This is achieved by applying a negative voltage through a Pt cathode which induces the electrolytic reduction of soil water oxygen. Initially the voltage induces an overestimate of the oxygen diffusion rate as all the oxygen in the soil water is reduced. After 3-10 minutes (Stolzy and Letey, 1964) a steady state is reached where the electrolytic reduction is proportional to the diffusion of oxygen from the gaseous phase to the liquid phase in soil. Once steady state is attained, the current which depolarises the electrode in response to the reduction of oxygen is directly proportional to the depolarising agent (the electrolytic reduction of oxygen). Since the main constraint to oxygen diffusion is the diffusion of oxygen from the gaseous phase (in air pockets) to the liquid phase (the soil water) it may be assumed that the electrolytic reduction of oxygen occurs at a rate comparable to the assimilation of a healthy plant root. A mathematical derivation of oxygen diffusion was central to Lemon's 1952 thesis (cited in Kowalik, 1985) where he defined oxygen diffusion as:

$$ODR = i.fx / a.n.F$$

Equation 6.3: For calculation of Oxygen diffusion rate ($g.cm^{-2}.min^{-1}$) where; a = Total surface area of the platinum electrode (cm), F = Faraday's constant, fx = Oxygen diffusion rate to the platinum electrode ($cm^{-1}.min^{-1}$), i = Measured current (amperes) and n = Number of electrons needed for the reduction of 1 molecule of oxygen (n = 4) from, Kowalik, (1985).

Stolzy and Letey (1964) appealed for the standardisation of the method in order to facilitate comparable measurements. The standard applied voltage for electrolytic reduction of oxygen is -0.65 V (below -0.2 V which oxygen is not reduced and above -0.8 V hydrogen is reduced). Subsequently Stolzy and Letey (1964), McIntyre (1970) and Kowalik (1985) all recommend that readings should be taken after a voltage of -0.65 V has been applied for over 3 minutes. Ordinarily the negative voltage is regulated by a variable resistor and measured using a voltmeter, in an arrangement similar to Figure 6.1.

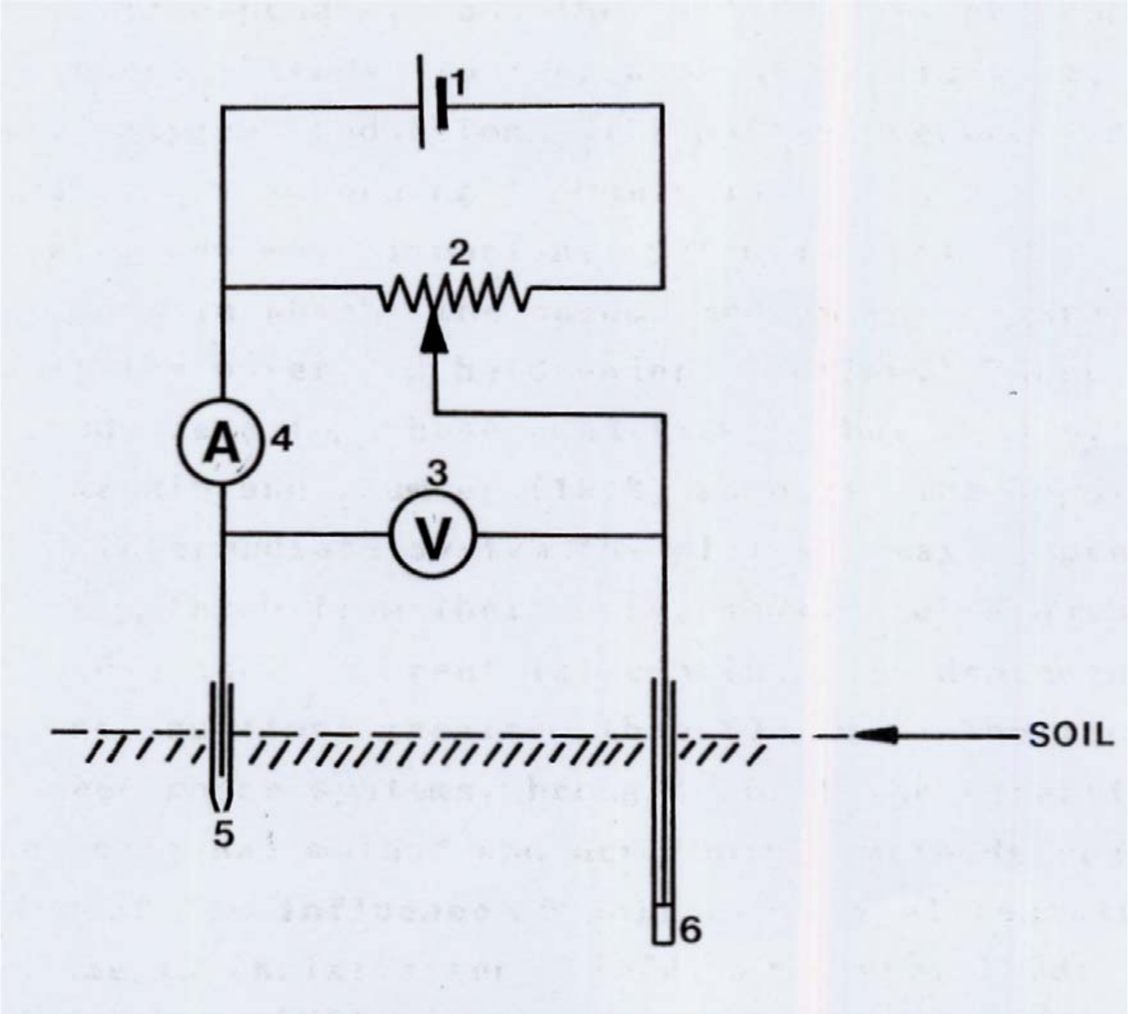

Figure 6.1: Conventional circuit to demonstrate the measurement of ODR, where; 1 = power source, 2 = variable resistor, 3 = voltmeter, 4 = ammeter, 5 = reference calomel electrode and 6 = the platinum electrode (from Humberto, 1991).

For evaluation of measured oxygen diffusion rates it is essential to categorise different rates. Initially Letey and Stolzy (1962) demonstrated that below 20 x 10^{-8} g.cm^{-2}.min^{-1} root growth was inhibited, however subsequently the inhibitory range has been further categorised (Kowalik, 1985) see Table 6.2. Despite such definite categorisation it is likely that the ODR responses are species specific.

Table 6.2: The three broad categories of plant response to Oxygen diffusion rate (taken from, Kowalik, 1985).

ODR $x10^{-8}$ *(g.cm^{-2}.min^{-1})*	*Response*
<20	Growth of many plants is inhibited
20-50	Some metabolic processes are inhibited
>50	Growth and development is normal

Numerous factors may influence an ODR measurement. Firstly those associated with the actual diffusion of oxygen into water such as temperature (Stolzy and Letey, 1964, McIntyre, 1970) and conductivity of water (Letey and Stolzy, 1962). Secondly those associated with the acquisition of the measurement such as poisoning of the cathode (Stolzy and Letey, 1964 and McIntyre, 1970) ensuring the cathode is fully immersed in water (Kowalik, 1985 and McIntyre, 1970) and electrode size (Stolzy and Letey, 1964). And finally those factors associated with the physiochemical properties of the soil such as organic matter content (Phene *et al.*, 1976), physical and chemical amendments (Warnaars *et al.*, 1970, Phene *et al.*, 1976, McIntyre, 1970), soil texture (Phene *et al.,* 1976) and aggregate size (Lemon and Erickson, 1954) also influence the measurement.

6.3 Objective

The following objective was fulfilled by the outlined experiments;

- To investigate internal soil physio-chemical conditions relative to different treatments.

6.4 Method

An orthogonal split plot experimental design was adopted for this investigation.

The lysimeters were split using a metal plate driven in with a sledgehammer. One lysimeter was investigated per day and all measurements where taken within 3 hours: the study was carried on 13th - 24th October 2001 and 1st - 30th Sept 2002.

6.4.1 Order of measurements

Measurements were taken at points marked on Figure 6.2. The order of each measurement is important. Within the category of each measurement a zigzag sampling strategy was adopted starting at point A1 (see Figure 6.2). Firstly redox measurements were taken rapidly after the slabs were exposed at a depth of 0.02 m from the exposed face. Afterwards the probe was wiped clean, oxygen diffusion rate was taken in a separate location finally EC and temperature were measured.

Soil for chemical analysis was taken from the central section of the lysimeters in both years of sampling, although in 2001 there were three zones and in 2002 five zones were differentiated.

6.4.1.1 Soil pH

Single soil samples for each zone per lysimeter destroyed were non-systematically selected for pH analysis. The soil was air dried and shattered using a hammer. Then ground using a centrifugal grinder (Glencreston/Retsch, Ball mill S100, Middlesex, UK) to pass through a 2 mm sieve. 30g of ground soil was mixed with 75 ml of distilled water in a beaker (2.5:1 ratio G Lovelace pers. com. 2004), stirred and left covered overnight. The following day the pH s of each solution was taken using an electronic pH meter (Oxford laboratories, 3020 pH meter). Firstly the pH meter was calibrated to different pH s using buffer solutions of known pH values. The calomel electrode was immersed in solution until measured pH and temperature had stabilised. When the measurement had been taken the electrode was rinsed with distilled water before insertion into the next sample.

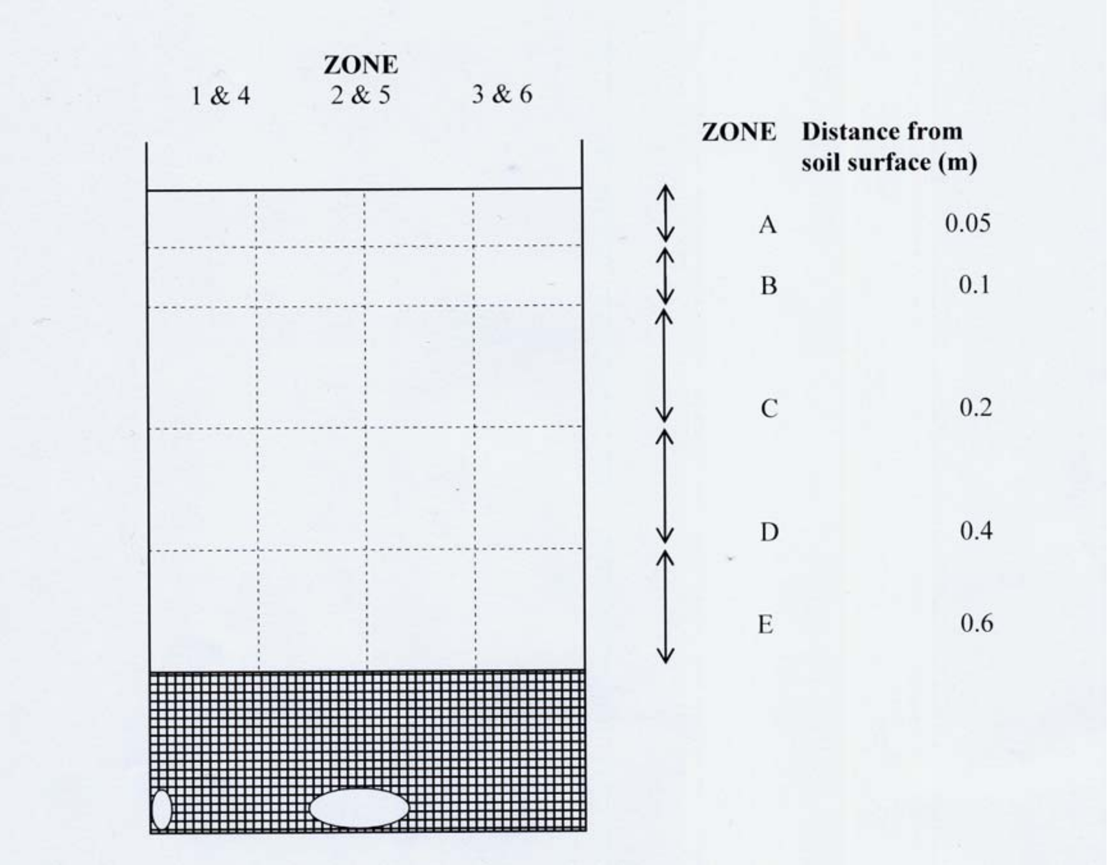

Figure 6.2: Plan of lysimeter showing sampling points.

6.4.2 Oxygen diffusion rate

Oxygen diffusion rate was measured in accordance to a method described by Kowalik (1985), where a negative voltage of -0.65 V was applied constantly through a cylindrical platinum electrode inserted to a depth of 0.03 m for five minutes. The same electrode was used throughout the study and therefore a conversion factor could be used (see appendix 9.4). The resultant resistance was measured using an ammeter (Master ranger TF2650, Marconi instruments, St Albans, UK). Subsequently ODR can be calculated from Equation 6.4.

$$\text{ODR} \quad = \quad 0.019.i\ (x10^{-8})$$

Equation 6.4: Conversion factor for the translation of measured μamps into ODR (g.cm^{-2}.min^{-1}).

Between each sample the Pt probe was wiped clean, in this study it was decided that the acquisition of measurements rapidly after exposure of the soil face was more important than ensuring fully reduced conditions of the Pt probe. ODR measurements were taken on a variety of soils after the lysimeter destruction experiment in order to provide comparative values.

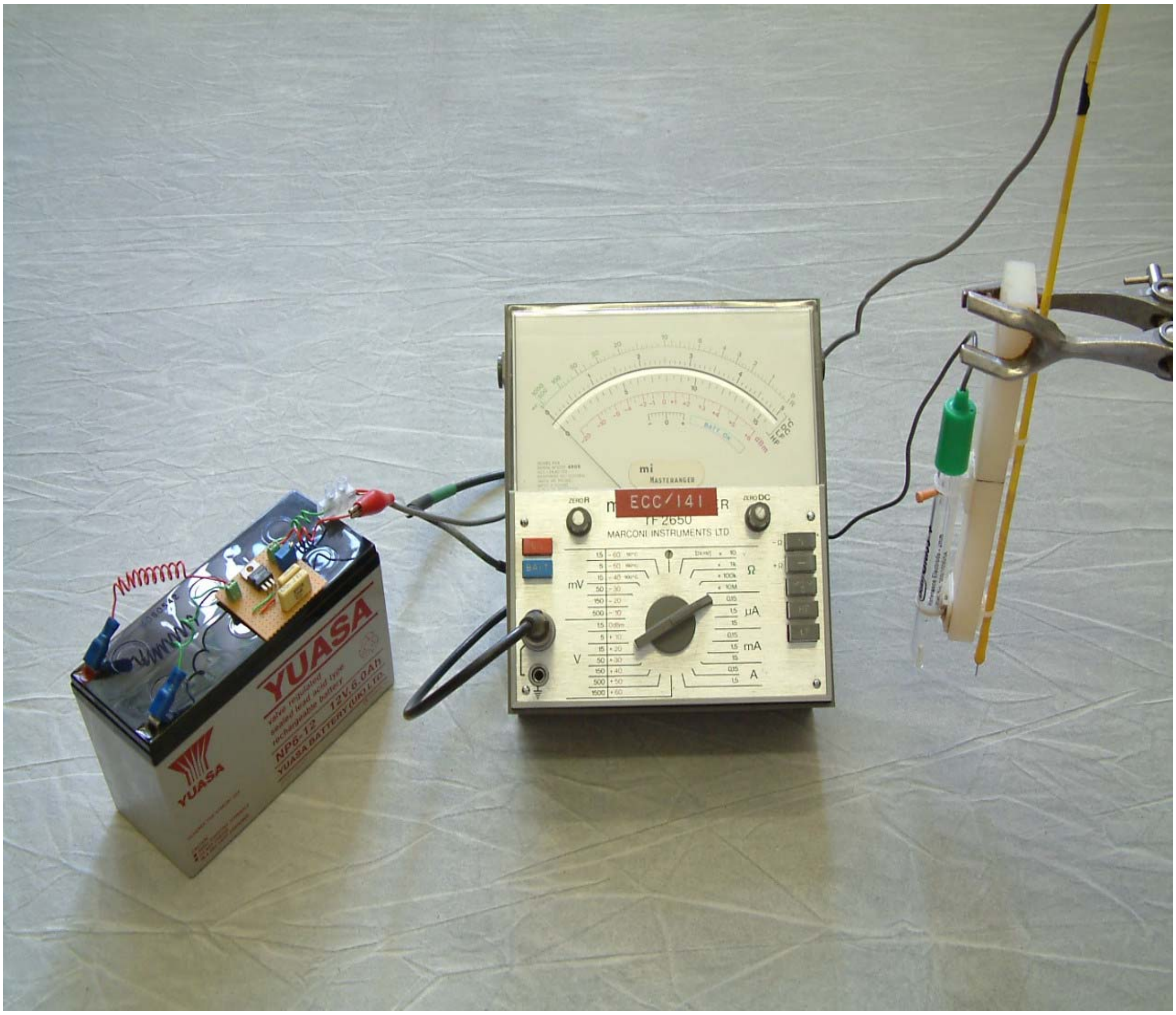

Figure 6.3: The setup used for ODR measurement, note the 12V DC 7Amphr Yuasa Battery, the master ranger ammeter and the platinum cathode and reference electrode on a block a constant distance apart.

6.4.3 EC and temperature

The EC and temperature were measured using a Sigmaprobe ECK-1 in the same way as detailed in Section 3.4.1 and retrospectively normalised to 20°C. The probe was inserted to a depth of 0.03 m from the exposed face of the split lysimeter and the measurement was repeated serially five times per location.

6.4.4 Redox

The redox potential (Eh_M) was measured using a platinum anode connected to a voltmeter (Whitegold, Precisiongold WC 020 range DC 2-200 mV) and a reference electrode (Calomel, BDH). The reference and platinum electrodes where kept a constant 0.03 m apart. The probe was inserted for three minutes then once steady state conditions occurred, the voltage was recorded once from the voltmeter. The Eh_M was then converted to Eh by accounting for the different potentials caused by the use of a calomel electrode using Equation 6.1 and normalised to pH using Equation 6.2.

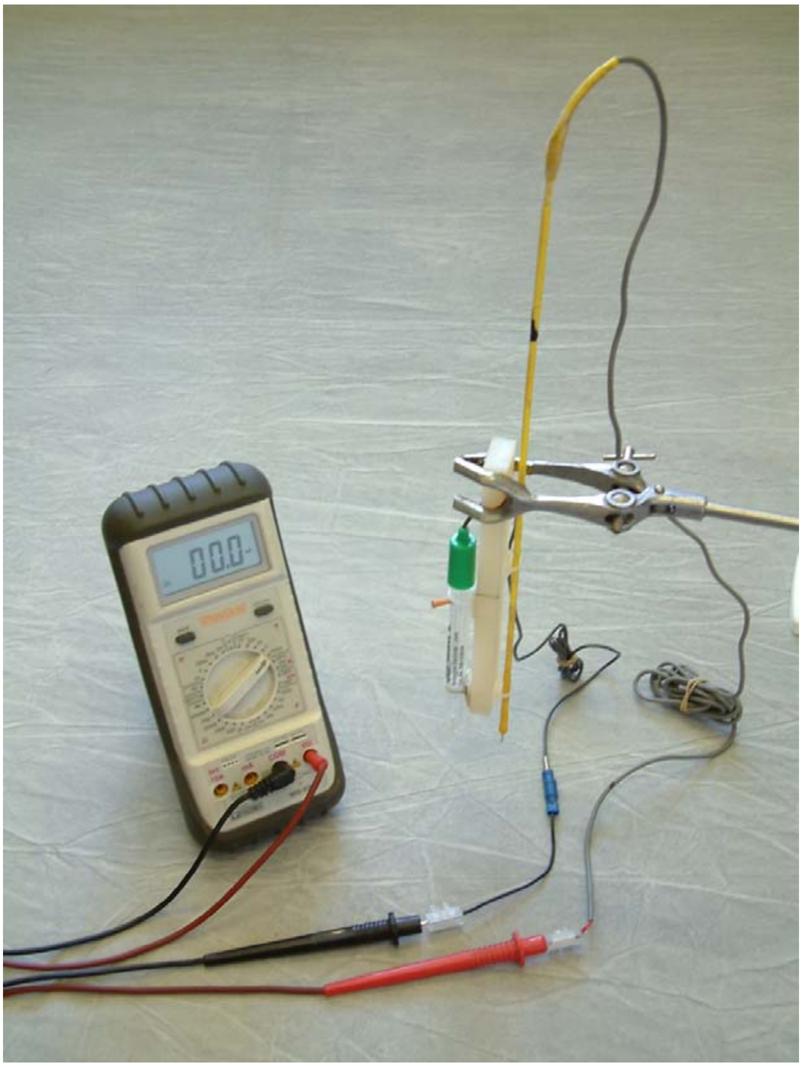

Figure 6.4: The redox arrangement employed for the analysis of internal lysimeter redox conditions, not the reference and Pt electrode are kept a constant distance apart.

6.4.5 Soil nitrogen determination

Soil sample “2” for each of the zones (A, B, C, D and E) was kept refrigerated (<14 days) until a portion was extracted for analysis of mineral based nitrogen (NO_{3-} and NH^{4+}) and water content.

Figure 6.5: A broken up lysimeter, note the middle section (sample 2 for each zone is harvested for collection of soil for chemical analysis; note the polypropylene sheath around the lysimeter.

20g +/- 0.05 g was weighed into a 125 ml bottle and 100 ml of 2M potassium chloride reagent was added to each bottle. The bottles were capped and shaken for two hours; at this point a blank determination was added. Once shaking was complete the samples were then filtered through a Whatman No. 4 filter paper and the filtrate was kept refrigerated until analysis (< 2 days). Determination was performed colourmetrically using a Burkard series 2000 segmented flow continuous analyser (Uxbridge, UK). The machine was calibrated using known standards, against which the soil extracts were determined (ammonium was measured within a wavelength of 650 nm and nitrate within a wavelength of 520 nm). Each sample was measured nine times. If dilution was required then de-ionised water used.

6.4.6 Soil chloride determination

A portion of Sample "2" for each of the zones (A,B,C,D and E) was dried, pounded with a hammer and then ground to pass through a 2 mm sieve using a centrifugal grinder (Glencreston/Retsch, Ball mill S100, Middlesex, UK). 15g +/-0.1 g of sample soil was deposited in a 100 ml bottle and 75 ml of deionised water was added. The mixture was shaken for 1 hour along with a blank and the resultant mixtures were filtered through a Whatman no. 4 filter paper. The filtrate was kept refrigerated until analysis (< 1day). The extract were analysed using a Techinicon autoanalyser (Technicon, EIRE). The individual samples were analysed colourmetrically (at a wavelength of 480 nm) relative to known standards. If dilution was required the de-ionised water was used.

6.4.7 The L1 treatment

An auxiliary treatment which received 75% of the leachate irrigation of the leachate only irrigation treatment was added in the 2002 experimental season. No physiological measurements were carried out on this treatment although by the end of the irrigation period five of trees were exhibiting total defoliation and the other 10 retained their leaves. This treatment was sub divided into two groups: L1a which denotes trees with relatively healthy above ground growth and L1b which denotes trees which demonstrate the epinastic response and defoliation.

6.5 Results

Table 6.3 shows that the leachate only irrigation treatment consistently has elevated chloride, ammonium and nitrate soil concentrations relative to the water only irrigation treatment in both years destructive analysis. However Table 6.3 also shows that in 2001 the concentrations of soil chloride, ammonium and nitrate are less than those measured in 2002 and ammonium and nitrate concentrations in the leachate only irrigation treatment are not significantly different from the water only irrigation treatment.

Table 6.3: Average nitrate, ammonium and chloride concentrations presented per lysimeter, obtained at the end of the irrigation period in 2001 and 2002. * = a negligible amount was determined; LSD's were determined using analysis of variance, where p = 0.05 and standard error of the mean is presented in brackets next to the concentration.

Treatment	*Average NO_3-N (mg/kg dry soil)*		*Average NH_4-N (mg/kg dry soil)*		*Average Cl (mg/kg dry soil)*	
	2001	*2002*	*2001*	*2002*	*2001*	*2002*
Water only	0.004 (*)	1.6 (0.97)	0.8 (0.08)	1.2 (0.6)	1841.6 (395.5)	1753 (283.7)
Leachate only	0.04 (0.01)	45.4 (16.5)	1.4 (0.3)	336.6 (77.4)	4519.3 (1119.3)	17918 (781.2)
LSD	**0.04**	**15.3**	**0.7**	**57.3**	**2615.7**	**2105.9**

6.5.1 Oxygen diffusion rate

The most striking output of the ODR data is that all the ODR measurements are low values (compare Table 6.2 and 6.6). Secondly although the values are low and the differences are small there is some statistically significant (LSD = 0.57 X 10^{-8}) differences between treatments.

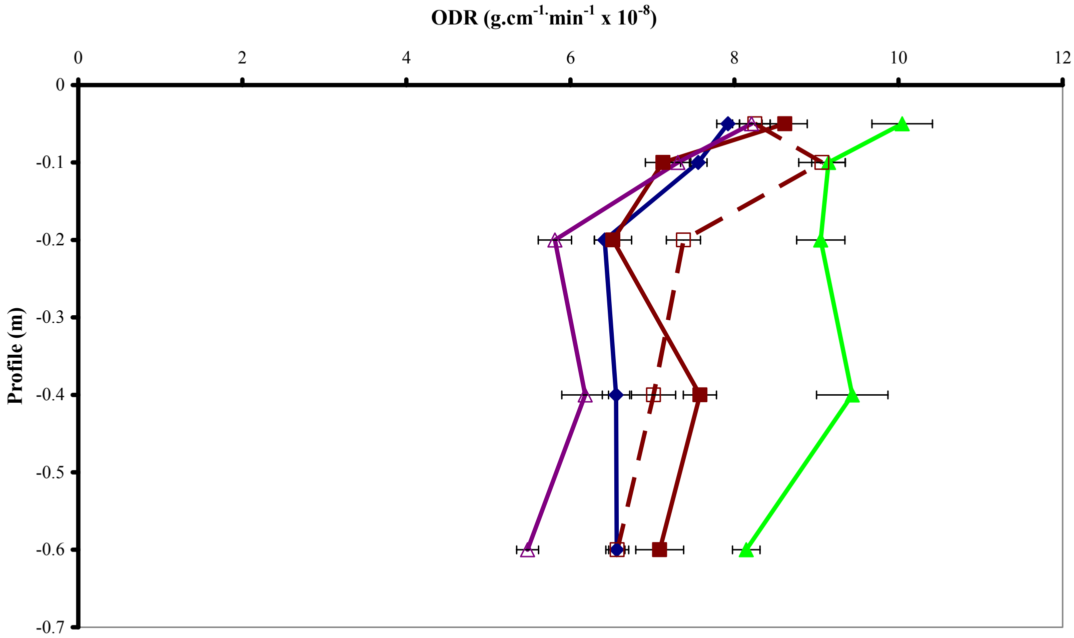

Figure 6.6: ODR profile within the longitudinally split lysimeters, measured at a depth of 0.03 m on the exposed face after 5 minutes of equilibration time.

The largest significant difference is between the cyclical water:leachate irrigation treatment (9.2 x 10^{-8}) and the leachate only irrigation treatment (6.6 x 10^{-8}). All treatments follow a broadly similar pattern of decreasing ODR with profile.

6.5.2 Redox potential

The redox potential measurements however demonstrate far less differentiation within the soil and therefore is not graphically presented. Average values are presented in Table 6.4 .

Table 6.4: The average redox potential per lysimeter for each treatment, in brackets is the SE mean, p = 0.05.

Treatment	*Average lysimeter redox potential (mV)*	
Water only	348.3	(25.2)
Leachate only	273.6	(14.6)
L1a	343.4	(19.9)
L1b	331.7	(10.1)
Cyclical leachate:water	381.4	(14.3)
LSD	**18.4**	

The average barrel redox of 381.4 mV for the cyclical water:leachate irrigation treatment was significantly higher than the other treatments. The leachate only treatment which had an average barrel redox of 273.6 mV was significantly lower than all the other treatments. The differences between the other treatments were not significant.

6.5.3 Electrical conductivity

The EC profile is less developed relative to the other physiochemical measurements. With the exception of the cyclical water:leachate irrigation treatment the profile indicates notable homogeneity within the lysimeter.

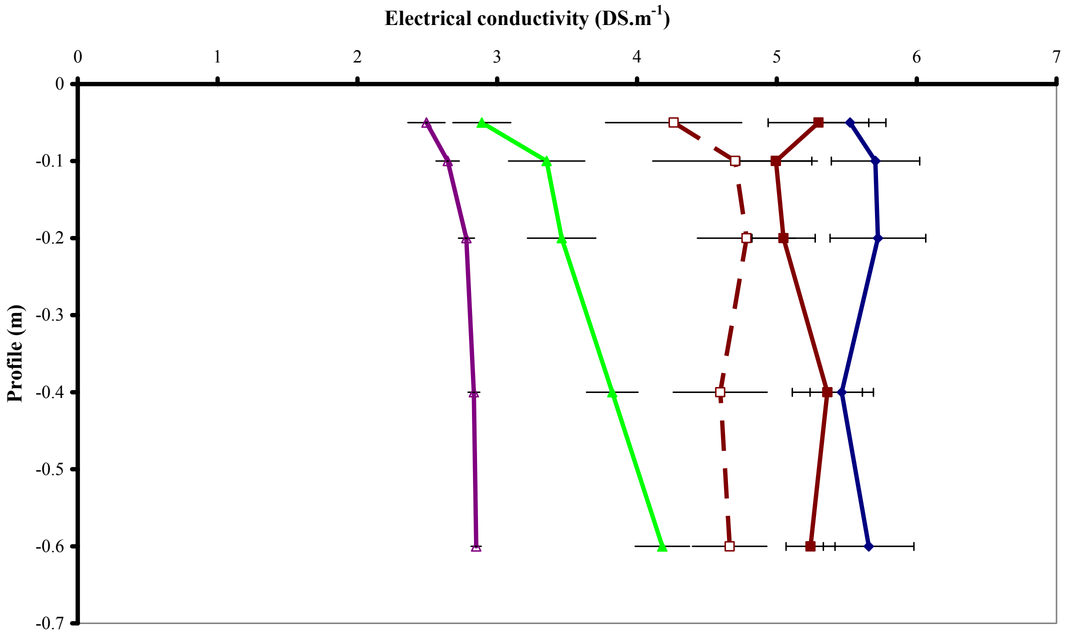

Figure 6.7: The EC at different points within the lysimeter profile for the water only (purple), cyclical leachate:water (green) L1a (brown dashed), L1b (brown solid) and leachate only (blue) irrigation treatments, the error bars are standard error of the mean.

Figure 6.7 and Table 6.4 indicate the average EC of all the treatments are significantly different. The leachate only treatment EC is over double the EC of water only and 1.6 times the EC of the cyclical leachate:water irrigation treatment. The L1 a and b treatments although statistically different (LSD = 1.15) to the leachate only treatment are nevertheless in a relatively similar range of EC as the leachate only treatment. Interestingly the L1 sub treatments demonstrate a maximum divergence in the upper (0.05 m) strata in measured EC with the L1a (which supported relatively healthier plants) less saline than L1b (which supported plants which demonstrated an epinastic response).

6.5.4 Soil nitrate

There is a notable difference (p = 0.05, LSD = 57.3) between the nitrogen impoverished water only treatment and all the other treatments.

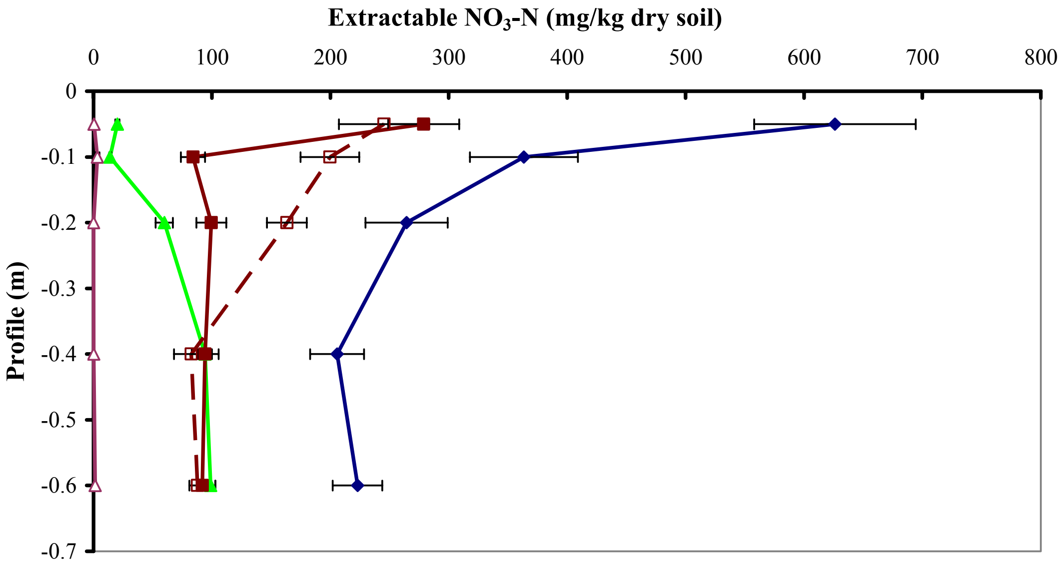

Figure 6.8: The vertical distribution of NO_3-N within the lysimeter profile for the water only (purple), cyclical leachate:water (green) L1a (brown dashed), L1b (brown solid) and leachate only (blue) irrigation treatments, error bars are SE mean

Furthermore both L1 a and b are notably different in profile and quantity from both the leachate only and the water only irrigation treatment. The cyclical water:leachate irrigation treatment demonstrates similarities with both the water only and L1 treatments. There is no significant difference between the cyclical water:leachate treatment and water only profile in the top 0.1 m but below that there are significant differences. In this surface layer there are differences between the L1a and L1b but below 0.4 m the profiles are the same.

6.5.5 Soil ammonium

There is significantly more (2-10 fold) ammonium in the leachate only irrigation treatment relative to all the other treatments, within the upper 0.4 m of the lysimeters. The cyclical leachate:water irrigation treatment demonstrates no significant difference from the control throughout the profile. L1 a & b are not significantly different from the water only or the cyclical water:leachate treatments at depths < 0.4 m ($p = 0.05$, LSD = 15.3). Below 0.4 m the level of ammonium is considerably more than the control.

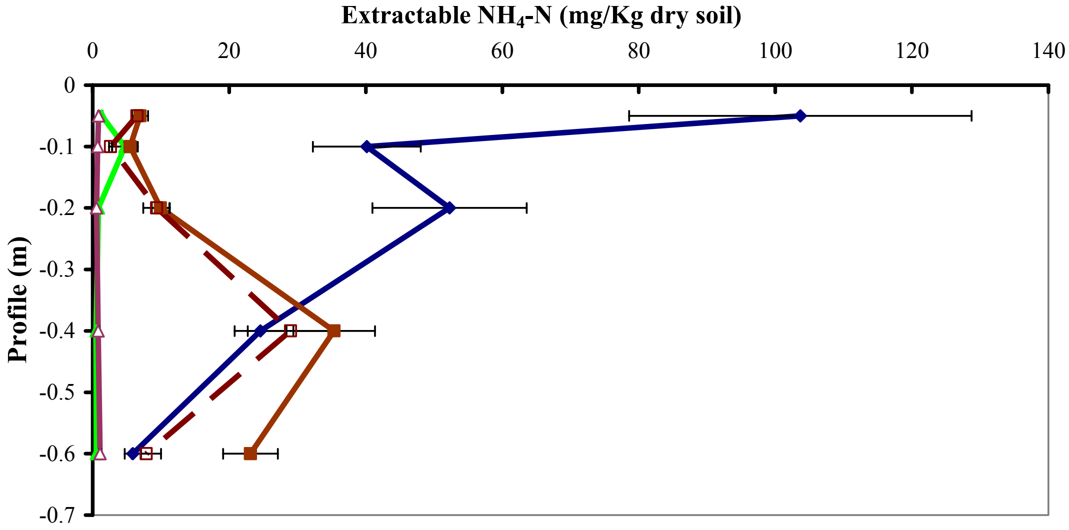

Figure 6.9: The vertical distribution of NH4-N within the lysimeter profile for the water only (purple), cyclical leachate:water (green) L1a (brown dashed), L1b (brown solid) and leachate only (blue) irrigation treatments, error bars are standard error of the mean.

6.5.6 Soil chloride

With the exception of the water only treatment there is a consistent trend of chloride accumulation in both the top and bottom of the lysimeter. The water only treatment has the lowest average lysimeter value 1753 mg Cl^-/kg dry soil. The leachate only and L1b treatment have 10 fold more chloride relative to the water only irrigation treatment. Finally the L1a treatment is significantly different (p = 0.05, LSD = 2105.9) to the L1b treatment both in average barrel value and profile, although the magnitude of the difference when compared with the magnitude of difference between the other treatments is relatively small except for the 0.05 m strata.

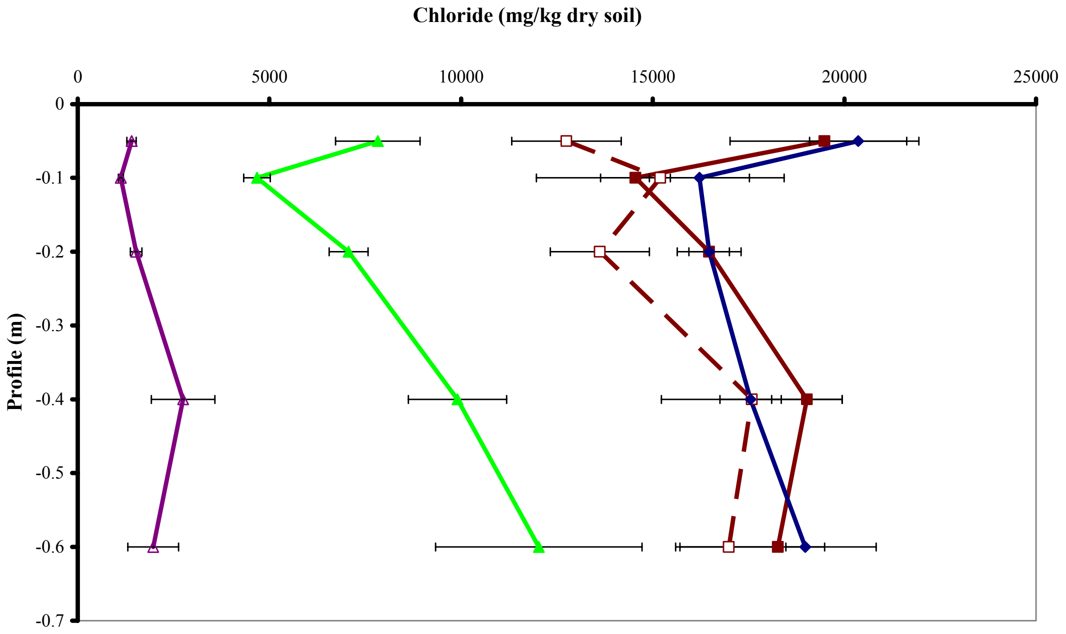

Figure 6.10: The vertical distribution of chloride within the lysimeter profile for the water only (purple), cyclical leachate:water (green) L1a (brown dashed), L1b (brown solid) and leachate only (blue) irrigation treatments, error bars are SE mean.

The cyclical water:leachate irrigation treatment has a chloride concentration intermediate between the water only and the leachate only and L1 treatments. Furthermore the cyclical water:leachate irrigation treatment demonstrates redistribution within the soil profile.

6.6 Discussion

Quantitative (e.g. leaf area) and qualitative measurements (e.g. epinastic response) influenced the timing and experimental protocol adopted in this section.

Consequently the results outlined in this section are a reflection of the edaphic conditions at the end of the experimental period. Nevertheless conclusions concerning the edaphic conditions can be drawn from the data.

There are a number of results which are indicative of a substrate which impedes gas and liquid movement: specifically ODR and redox potential (Table 6.4) and distribution of chloride (see Figure 6.10) and EC (see Figure 6.7) in all leachate treatments. It is however possible that the ODR readings are low because of the adopted protocol. The rapid acquisition of ODR measurements *in situ* was considered to reflect the true ODR of the soil rather than extrication and measurement under laboratory conditions, despite not ensuring the reduction of the Pt cathode. The distribution of chloride in the treatments is undermined by sampling the central portion of the lysimeter, but because of the concurrent EC data one can assert that chemicals are not easily distributed throughout the lysimeter. One possible reason for this is that the high bulk density of the Oxford clay used poses an inherent constraint to both the movement of ions and gas.

From Figure 6.8, **Figure 6.9** and Table 6.3 it is evident that nitrification processes are happening within the lysimeters. Considering the results presented in Chapter 4 and Chapter 5 one can expect that some of the converted nitrate was sequestered by *Salix viminalis*. However from Figure 6.8 it is apparent that Nitrate is easily lost from the lysimeter. The proportions removed by plant uptake, soil leaching or gaseous losses is undetermined and therefore requires further investigation.

The chemical distribution of the salts along the profile of the sampled lysimeters is very interesting. Firstly the water only irrigation treatment demonstrates characteristics of a soil which has a relative lack of salts compared to the other treatments. Secondly the nitrate and ammonium concentrations in the 0-0.4 m depths in the L1 treatment are not significantly different, but differences between chloride concentrations in this zone are significantly different, this indicates not only the sequestration of these nitrogenous compounds, but the deleterious effect of chloride in this zone. The distribution of the nitrogenous compounds in the L1 treatment may be indicative of a relative abundance of roots structures in the upper strata; this has also been found in Souch *et al.* (2004). This is of particular importance when one considers that the potential for root penetration of the engineering is one of the major drawbacks to the growing of trees on landfill caps (Dobson and Moffat, 1993). Finally the cyclical water:leachate irrigation treatment in 2002 shows that periodic flushing with water results in a relative attenuation of nitrogenous compounds (see Figure 6.8 and **Figure 6.9**) as does data obtained in 2001 (Table 6.3).

Despite the results in this section being indicative of a soil which impedes liquid and gas movement it also shows that if plants are irrigated with leachate beyond its maximum capacity (like the leachate only irrigation treatment) then they are likely to run the risk of accumulation of nitrogenous compounds which presumably over a period of soil water excess normally the winter months would leach into groundwater. This is substantiated from the results obtained in 2001 and from the cyclical water:leachate treatment. Quantification of salt washout in response to different irrigation regimes and soil types is strongly recommended for future investigation.

6.7 Conclusions

From the data obtained *via* lysimeter destruction at the end of the irrigation period one can conclude that the compacted Oxford clay used in this study was inhibitory to chemical movement, consequently under leachate irrigation regimes salts are liable to accumulate although prolonged periods of irrigation with water, or no irrigation at all resulted in a relative “wash out” of salts. Furthermore the inhibition of chemical movement also affected Oxygen diffusion rates and redox potential; consequently the *S. viminalis* in this study were growing in substrate which under current classification structure can be defined as inhibitory to normal plant growth.

7 Conclusions and further recommendations

The use of SRC as a landfill leachate attenuation system offers a number of enticing benefits most notably the attenuation of leachate mass (Brierley *et al.*, 2002) and nitrate (Aronsson and Bergstrom, 2001) and the enhancement of biomass (Ettala, 1988). However the correlation with plant morphological and physiological traits with leachate irrigation highlights the necessity for an appropriate gauge to monitor leachate application.

Stephens *et al.* (2001) concluded that chloride may be the main constraint to a landfill leachate attenuation system. Consequently EC was employed to monitor edaphic conditions under leachate irrigation. However instantaneous EC was found to be an unsatisfactory measure due to a plateauing of EC in the leachate only irrigation treatment and because leachate irrigation appeared to instigate chronic rather than acute stress. Contemporary experimental studies e.g. WRC (2002) have also employed electrical conductivity but irregular measurements have may have masked the impact of prolonged leachate application on soil EC.

Without a standardised measure of the stress imposed by leachate irrigation cross comparison between studies is difficult, compounded by vague descriptions of irrigation schedules and leachate composition. Consequently the adoption of EC days for further investigation is strongly recommended as it provides a relatively accurate description of salt loading (see Figure 3.8) and soil matric potential (see Figure 3.9) and is correlated with leaf area (see Figure 4.4) and foliar gas exchange (see Figure 5.6 and Figure 5.7). It is noteworthy that Delta-T has a new product in development which incorporates a temporal element into the expression of EC in addition to the raw format. However, one advantage of EC days which was overlooked by Delta-T is incorporation of a baseline EC, which is central to the determination of EC days, this permits accurate interpretation of the salt addition to a soil without interference of the soils inherent baseline EC.

Despite the results of this study and Stephens *et al.* (2001) it is pertinent that other constituents of leachate may be more potent constraints to plant growth. For example herbicide presence, was found by WRC (2002) may have a devastating effect on a landfill leachate attenuation system. Furthermore, heavy metals are frequently found in landfill leachate. In addition, Cureton *et al.* (1991) concluded that leachate irrigation results in a reduction of hydraulic conductivity, which coupled with a possible soil water excess resulting from improperly managed leachate irrigation could result in anaerobic edaphic conditions. This highlights not only the need for accurate quantification of edaphic conditions but screening of applied leachate.

One of the most positive outcomes of EC days is the correlation between EC days and gas exchange parameters. A relative abundance of roots in the top 0.2 m of the lysimeters is likely, data presented in Chapter 6 (e.g. Figure 6.9) supports this; fine roots are the principal sites of ABA production and ABA has an effect on gas exchange through controlling stomata (Taiz and Zeiger, 1998). Possible reasons for the predominance of roots in a small volume of soil could stem from the experimental setup. The topsoil incorporated Biogran^{TM} for improved structure and nutrition. Consequently the zone was less compacted than the subsoil and this may predispose the proliferation of roots relative to the compacted zone beneath. Furthermore, the availability of nitrogen addition at an early stage of growth induces fine root growth

(Aronsson and Bergstrom, 2001). Access to a low volume of exploitable soil often results in a restriction in growth (Liang *et al.,* 1999). Chapter 4 shows stimulation of biomass from leachate application yet section 5.5.1.3 shows that an initially higher degree of carboxylation in the leachate only treatment relative to the water only irrigation treatment is suppressed with leachate irrigation. Results in Chapter 6 also indicate that soil compaction had a profound effect on soil physio-chemical properties. Principally it was found that the substrate, especially in the lower zones of the lysimeters impeded liquid and gas movement. On one hand this is a positive result for landfill leachate attenuation system as it indicates a reduced propensity for salt washout, although on the other hand it highlights the necessity for avoiding excess application. Nevertheless a “mass balance” experiment which investigates the effect of salt washout under a variety of irrigation regimes and possibly soil and plant materials would be a seminal work within the idea of a landfill leachate attenuation system.

A mass balance experiment should incorporate detailed investigations into the foliar sequestration of salt inherent to leachate. There are still unanswered questions resulting from this study which relate to the accentuated gas suppression under leachate irrigation (see Figure 5.8 and Figure 5.9) and the initial elevated gas exchange at the commencement of irrigation in the second years treatment (see Figure 5.6 and Figure 5.7).

Finally this study shows that *Salix viminalis* is capable of growth in Oxford clay soil whilst being heavily irrigated (see Section 2.4.1) with a landfill leachate with a relatively high chloride concentration (see Table 2.1). Future investigations should favour plant material which is both tolerant of low O_2 soil conditions and capable of withstanding elevated soil matric potentials. There should be future investigation into this technology because the *Salix viminalis* in this study demonstrated enhanced morphological traits in response to landfill leachate irrigation, which would be beneficial to a landfill operator i.e. a two fold enhancement of dry weight. An EC days of 200 $DS.m^{-1}.day$ should not be exceeded because of the chronic stress exhibited by *Salix viminalis* after this point. However the results from this study coupled with the results from an investigation into the fate of chloride in the system may facilitate the implementation and management of a commercial landfill leachate attenuation system.

8 References

Adams P, Thomas J C, Vernon D M, Bohnert H J and Jensen R G (1992) Distinct cellular and organismic responses to salt stress. *Plant Cell Physiology*, 33, 1215-1223.

Aronsson P G and Bergstrom L F (2001) Nitrate leaching from lysimeter grown short rotation willow in relation to N-application, irrigation and soil type. *Biomass and Bioenergy*, 21, 155-164.

Bassil E S and Kaffka S R (2002) Response of safflower to saline soils and irrigation on consumptive water use. *Agricultural Water Management*, **54**, 67-80.

Baum C, Weih M, Verwijst T and Makeschin F (2002) The effects of nitrogen fertilisation and soil properties on mycorrhizal formation of *Salix viminalis*. *Forest Ecology and Management*, **160**, 35-43.

Bednarz C W and van Iersel M W (1998) Semi continuous carbon dioxide exchange rates in cotton treated with commercially available plant growth regulators. *The Journal of Cotton Science,* **2**, 136-142.

Belkohodja R, Morales F, Abadia A, Gomez-Aparisi J and Abadia J (1994) Chlorophyll fluorescence as a possible tool for salinity tolerance screening in Barley (*Hodeum vulgare* L.). *Plant Physiology,* **104,** 667-672.

Bethan-Falvay G, Andrade G, Azcon-aquliar C (1997) Plant and soil response to mycorrhizal fungi and rhizobacteria in nodulated or nitrate-fertilized peas. *Biology of. Fertilised Soils,* **24**, 164-168.

Bjorkman O and Powles S B (1984) Inhibition of photosynthetic reactions under water stress: interaction with light level. *Planta*, **161**, 490-504.

Bjorkman O, Demmig B and Andrews T J (1988) Mangrove photosynthesis: response to high irradiance stress. *Australian Journal of Plant Physiology,* **15,** 43-61.

Blackman P G and Davies W J (1985) Root to shoot communication in maize plants of the effects of soil drying. *Journal of Experimental Botany*, **36**, 39-48.

Boone R, Nadelhoffer K, Canary J and Kay J (1998) Roots exert a strong influence on the temperature sensitivity of soil respiration. *Nature*, **396**, 570-572.

Boyer J S (1995) Measuring the water status of plants and soils. Academic Press.

Brierley E D R, McDevitt J E, Thorn P, Tyrrel S F and Stephens W (2001) Application of landfill leachate to willow short rotation coppice. *Aspects of applied biology 65*, *Biomass and energy crops II*.

Calatayud A, Rameirez J W, Iglesias D J and Barreno E (2002) effects of ozone on photosynthetic CO_2 exchange chlorophyll a fluorescence and antioxidant systems in lettuce leaves. *Physiologia Plantarum*, **116**, 308-311.

Campbell R and Seaborn G (1972) Yield of flue cured tobacco and levels of soil oxygen in lysimeters with different water table depths. *Agronomy Journal*, **64**, 730-733.

Carvalho L S, Osorio M L, Chaves M M and Amancio S (2001) Chlorophyll fluorescence as an indicator of photosynthetic functioning of *in vitro* grapevine and chestnut plantlets under *ex vitro* acclimatization. *Plant Cell, Tissue and Organ culture,* **67,** 271-280.

Chan G Y S and Wong M H (2002) Revegetation of landfill sites. *Encyclopaedia of Soil Science*, 1161-1166.

Choisel G (1997) Effect of water stress on growth and dry matter production of willow cuttings. MSc thesis, Cranfield University at Silsoe.

Clendenen G (1996) Use of harmonised equations to estimate above ground woody biomass for hybrid poplar clones in the Pacific Northwest. *Biomass and Bioenergy*, **11**, 475-482.

Corseuil H X and Moreno F N (2001) Phytoremediation potential of willow trees for aquifers contaminated with ethanol blended gasoline. *Water Research,* **35**, 3013-3017.

Cureton P M, Groenevelt P H and McBride R A (1991) Landfill leachate recirculation: effects on vegetation vigour and clay surface cover infiltration. *Journal of Environmental Quality,* **20,** 17-24.

Dahnke W C and Whitney D A (1988) Measurement of soil salinity. Recommended chemical soil tests procedures for the north central region. *North Dakota Agricultural experimental station Bulletin 499.*

Darayan S, Liu C, Shen L C and Shattuck D (1998) Measurement of electrical properties of contaminated soil. *Geophysical Prospecting,* **446**, 477-488.

Dasberg S and Bakker J (1970) Characterising soil aeration under changing soil moisture conditions for bean growth. *Agronomy Journal*, **62**, 689-692.

De Jong E, Ballantyne A, Caneron D and Read D (1979) Measurement of apparent electrical conductivity of soils by electromagnetic induction probe to aid salinity surveys. *Soil Science Society of America Journal*, **43**, 810-812.

De Pascale S and Barbieri G (1995) Effects of soil salinity from long term irrigation with saline sodic soil water on yield and quality of winter vegetables. *Scientia Horticulturae*, **64**, 145-157.

De Pascale S and Barbieri G (1997) Effects of soil salinity and top removal on growth and yield of broadbean as a green vegetable. *Scientia Horticulturae*, **71**, 147-165.

Delfine S, Alvino A, Villani M C and Loreto F (1999) Restrictions to carbon dioxide conductance and photosynthesis in Spinach leaves recovering from salt stress. *Plant Physiology*, **119**, 1101-1106.

Dellaville N B (1992) Determination of the soil-paste pH and conductivity of saturation extract. In Handbook reference methods for soil analysis. *Soil and plant analysis council Inc Athens GA.*

Delta-T (2001) User manual for the Sigmaprobe ECK-1.Delta-T devices, Cambridge.

Dimtriou I and Aronsson P J (2004) Nitrogen leaching from short rotation coppice after intensive irrigation with wastewater. *Biomass and Bioenergy,* **26**, 433-441.

Dobson M and Moffat, A J (1993) The potential of woodland establishment on landfill sites. *Department of the environment HMSO.*

Donovan L A, Linton M J and Richards J H (2001) Predawn plant water potential does not necessarily equilibrate with soil water potential under well water conditions. *Oecologia,* **129**, 328-335.

Dowlen N (1997) The response of willow trees to irrigation with landfill leachate. *MSc thesis, Cranfield University at Silsoe.*

Drommerhauser D, Radcliffe D, Brune D and Gunter (1995) Electromagnetic conductivity surveys of dairies for groundwater nitrate. *Journal of Environmental Quality,* **24**, 1083-1091.

Durr, S (1996) The response of biomass in coppiced trees to differing concentrations of landfill leachate. *MSc thesis, Cranfield University at Silsoe.*

Elowson S (1999) Willow as a vegetation filter for cleaning of polluted drainage water from agricultural land. *Biomass and Bioenergy*, **16**, 281-290.

Ettala M O (1988) Short rotation tree plantations at sanitary landfills. *Waste Management and Research*, **6**, 291-302.

Evans J R (1983) Nitrogen and photosynthesis in flag wheat (*Triticum aestivum).* *Plant physiology*, **72**, 297-302.

Gambrell R, and Patrick W (1978) Chemical and microbiological properties of anaerobic soils and sediments. *In Hook D and Crawford M, Plant life in anaerobic environments. Annals of Arboricultural Science*. 375-423.

Glinski, J., and Stepniewski W (1985) Soil Aeration and Its Role for Plants, *CRC Press, Boca Raton, Florida*.

Goor F, Davydchuk V and Ledent J-F (2001) Assessment of the potential of willow SRC plants for energy production in areas contaminated by radionuclide deposits: methodology and perspectives. *Biomass and Bioenergy*, **21**, 225-235.

Grobbelaar J and Mohn (2002) ozone stress in rust resistant and susceptible *Helianthum annus* cultivars as measured with chlorophyll fluorescence. *South African Journal of Botany,* **61**, 469-474.

Hall R L, Allen S J, Rosier P T W, Smith D M, Hodnett M G, Roberts J, Hopkins R, Davies H, Kinniburgh D G and Gooddy D C (1996) Hydrological effects of short rotation forestry. *NERC. ETSU B/W5/00275/REP*.

Harrington D W and Marris P J (1986) The treatment of leachate: A UK perspective. *Water pollution Control*, **85**, 45-55.

Hartley, SE and Jones CG (1997) Plant chemistry and herbivory, or why the world is green. *Ch. 10 in Crawley MJ (ed) Plant Ecology. (2nd Ed.) Blackwell Science.*

Hartsock N J, Mueller T G, Thomas G W, Barnhisel R I, Wells KL and Shearer S A (2002) Soil electrical conductivity variability. *Proceeding of the 5th international conference on precision agriculture.* Http://www.bae.uky.edu/~precag/PrecisionAg/reports/soil_EC_Variabilty.

Hillel D (1998) Environmental soil physics. *Academic press.*

Humberto O M F (1991) Oxygen transport in tilled clay soils. *Ph.D Thesis – Cranfield University, Silsoe.*

Ilies P and Mavinic D S, (2001) The effect of decreased ambient temperature on the biological nitrification and denitrification of a high ammonia landfill leachate. *Water Research,* **35,** 2065-2072.

Kaiser W M, Kaiser G, Prachuab P K, Wildman S G and Heber U (1981) Photosynthesis under osmotic stress. *Planta*, **153,** 416-422.

Katerji N, van Hoorn J, Hamdy A and Mastrorilli M (1992) Effect of saline water on soil salinity and water stress, growth and yield of several crops. *International Conference on Land Water Research Management in the Mediterranean region, (Bari) Italy 4-8 Sept pp 357-381.*

Keck R W and Boyer J S (1974) Chloroplast response under low leaf water potentials. *Plant Physiology*. **53,** 474-479.

Keller C and Frischnecht F (1966) Electrical methods in geophysical prospecting. *Pergamon Oxford, 519.*

King D (1969) Soils of the Luton and Bedford district. Agricultural Research Council soil survey. Special; survey No. 1.

Kowalik P J (1985) Influence of land improvement on soil oxidation. *Swedish University of Agricultural sciences. Report 42.*

Kramer P and Boyer J S (1995) Water relations of plants and soils. *Academic press Inc.*

Latiri-Souki K, Nortcliff S and Lawlor D W (1998) Nitrogen fertiliser can increase dry matter, grain production and radiation and water use efficiency for Durum wheat under semi arid conditions. *European Journal of Agronomy*, **9**, 21-34.

Lebourgeois F, Levy G, Aussenac G, Clerc B, Willm F (1998) Influence of soil drying on leaf water potential, photosynthesis, stomatal conductance and growth in two black pine varieties. *Annals of Science Forestry*, **55**, 287-299.

Lefebvre X, Lanini S, Houi D (2004) The role of aerobic activity on refuse temperature rise, I. Landfill experimental study. Waste Management and Research, **5**, 444 – 452.

Lemon E and Erickson A (1952) The measurement of oxygen diffusion in soil with a platinum microelectrode. *Soil Science American proceedings*, **15**, 160-163.

Letey and Stolzy (1962) Measurement of oxygen diffusion rates with a platinum electrode, theory and equipment. *Hilgardia*, **35** 545-576

Levitt J, (1980) Responses of plants to environmental stresses, ***2nd ED.*** *Academic press, New York.*

Liang J, Zhang J, Chan G Y S and Wong M H (1999) Can differences in root responses to soil drying and compaction explain differences in performance of trees growing on landfill sites? *Tree Physiology*, **19**, 619-624.

Lichtenthaler H K (1995) Vegetation stress: An introduction to the concept of stress in plants. *Journal of plant physiology,* **148,** 4-14.

Lindegaard K N, Parfitt R I, Donaldson G, Hunter T (2001) Comparative trials of elite Swedish and UK biomass willow varieties, *Biomass and Energy Crop II. Aspects of Applied biology 65*,

Lindroth P and Cienciala E (1996) Water use efficiency of short rotation *Salix viminalis* at leaf tree and stand scales. *Tree Physiology*, **16**, 257-262.

Loreto F, Centritto M and Charzoulakis K (2003) Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. *Plant cell and Environment*, **26,** 595-601.

Lowenstein N J and Pallardy S G (1998) Drought tolerance, xylem sap ABA and stomatal conductance during soil drying: a comparison of young plants of four temperate deciduous angiosperms. *Tree Physiology*, **18**, 421-430.

MAFF (1984) Fertiliser recommendations for agriculture and horticultural crops. *Reference Book 209, HMSO London.*

MAFF (1986) The analysis of agricultural materials. *Ref Book 427, 3rd ED HMSO London.*

Martin P J, Brierley E D R, McDevitt J E, Moffat A J, Stephens W, Tubby I and Tyrrel S F (2002) Biomass production on landfill sites. A report to Shanks^{TM}.

Maurice C, Ettala M and Lagerkvist A (1998) Effects of leachate irrigation on landfill vegetation and subsequent methane emissions. *Water, Air and Soil Pollution*, **113,** 203-216.

Maxwell K and Johnson G N (2000) Chlorophyll fluorescence – a practical guide. Journal of Experimental Botany, **51**, 659-668.

McIntyre D (1970) The platinum microelectrode method for soil aeration measurement. *Advances in Agronomy*, **22**, 235-283.

McNeill, J.D (1980) Electrical conductivity of soils and rocks. *Technical Note TN-5. Geonics Ltd., Mississauga, ON.*

Monteith J L (1977) Climate and the efficiency of crop production in Britain. *Philosophical Transactions of the Royal Society B*, **281**, 277-294.

Nelson P N and Ham G J (2000) Exploring the response of sugar can to sodic and saline conditions through natural variation in the field. *Field Crops research*, **66**, 245-255.

Nobes D (1996) Troubled waters: Environmental applications of electrical and electron methods. *Surveys in Geophysics,* **17,** 393-454.

O'Toole J C, Crookston R K, Treharne K J and Ozbun J L (1976) Mesophyll resistance and carboxylase activity. *Plant Physiology*, **57,** 465-468.

Ogren E (1988) Photoinhibition of photosynthesis in willow leaves under field conditions. *Planta*, **175**, 229-236.

Ogren E (1988) Suboptimal nitrogen status sensitizes the photosynthetic apparatus in willow leaves to long term but not short term water stress. *Photosynthesis Research*, **18,** 263-275.

Ogren E (1990) Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. *Plant Physiology*, **93**, 1280-1285.

Ogren E and Oquist G (1985) Effects of drought on photosynthesis, chlorophyll fluorescence and photoinhibition in intact willow leaves. *Planta*, **166**, 380-388.

Ogren E and Sjostrom M (1990) Estimation of the effect of photoinhibiton on the carbon gain in leaves of a willow canopy. *Planta*, **181**, 560-567.

Percival G C and Galloway A (1999). The potential pf chlorophyll fluorescence measurements to detect salt and water logging stress in urban trees. *Acta Horticulturae,* **496**, 253-260.

Perttu K L (1998) Environmental justification of short rotation forestry in Sweden. *Biomass and Bioenergy*, **15:1**, 1-6.

Perttu K L and Kowalik P J (1997) *Salix* vegetation filters for purification of water and soils. *Biomass and Bioenergy,* **12:1**, 9-19.

Peterson T A, Blackmer T A, Francis D D and Schepers J S (2002) Using a chlorophyll meter to improve N management. *Nebguide, Cooperative extension, Institute of agriculture and natural resources, University of Nebraska-Lincoln.* Http://www.ianr.unl.edu/pubs/soil/g1171.htm.

Phene C, Campbell R and Doty C (1976) Characterization of soil aeration *in situ* with automated oxygen diffusion measurements. *Soil science*. **122**, 271-281.

Pinheiro C, Chaves M M and Ricardo C P (2001) Alterations in carbon and nitrogen metabolism induced by water deficit in the stems and leaves of *Lupinus albus*. *Journal of Experimental Botany*, **53**, 1063-1070.

Proe M F, Craig J, Griffiths J Wilson A and Reid E (1999) Comparison of biomass production in coppice and stem woodland management systems on an imperfectly drained gley soil in central Scotland. *Biomass and Bioenergy*, **17**, 141 151.

Ramkelawan E and Braithwaite R (1990) Leaf area estimation by non destructive methods in sour orange (*Citrus aurantium L*). *Tropical Agriculture*, **67**, 203-206.

Raven J A and Sprent J I (1993) Nitrogen assimilation and its role in plant water relations. In Smith J and Griffiths (Eds) (1993) "Water deficits: Plant responses from cells to community". Oxford Bios Scientific publishers Ltd.

Rhoades, J.D. and D. L. Corwin. (1981) Determining soil electrical conductivity-depth relations using an inductive electromagnetic soil conductivity meter. *Soil Science Society of American Journal*, **45**, 255-260.

Robbins N and Pharr D (1987) Leaf area prediction models for cucumber from linear measurements. *Hortiscience*, **22**, 1264-1266.

Robinson D A, Gardner C M K and Cooper J D (1999) Measurement of relative permittivity in sandy soil using TDR, Capacitance and Theta probes: comparison, including the effects of bulk soil electrical conductivity. *Journal of Hydrology*, **223**, 198-211.

Rowell D (1981) Oxidation and reduction. *In Greenland D and Hayes M eds "the chemistry of soil processes.* Wiley Chichester UK.

Rowell D (1988) Flooded and poorly drained soils. *In Wild A, "Soil conditions and plant growth".* Longman scientific and technical. UK 899-926.

Sellin A (1996) Base water potential of *Picea abies* as a characteristic of the soil water status. *Plant Soil*, **184**, 273-280.

Shangguan Z P, Shao M A and Dyckmans J (2000) Nitrogen nutrition and water stress effects on leaf photosynthesis gas exchange and water use efficiency in winter wheat. *Environmental and Experimental Botany,* **44**, 141-149.

Shimishi D (1970) The effect of nitrogen supply on some indices of plant water elations of beans. *New Phytologist*, **69,** 413-424.

Shrive S C, McBride R A and Gillespie T J (1990) Physiological and spectral responses of sugar maple (*Acer saccharum*) to MSW leachate spray irrigation. *Waste Management and Research,* **8,** 3-19.

Sifola M I and Postiglione L (2002) The effect of increasing NaCl in irrigation water on growth, gas exchange and yield of tobacco Burley type. *Field Crop Research,* **74,** 81-91.

Smith S and Read D J (1997) Mycorrhizal symbiosis. *2nd Ed. Academic press*

Souch C A (1996) Water use and productivity in three poplar clones. *PhD thesis, Cranfield University at Silsoe.*

Souch C A, Martin P J, Stephens W and Spoor G (2003) Effects of soil compaction and mechanical damage at harvest on growth and biomass production of short rotation coppice willow. *Plant and soil.*

Soule J and Malcolm J, (1970) A simple method for estimating mango leaf areas. *Proceedings of the Tropical Reg. American. Society for Horticultural Science*, **14**, 84-88.

Steduto P, Albrizio R, Giorio P and Sorrentino G (2000) Gas exchange and stomatal and non stomatal limitations to carbon assimilation of sunflower under salinity. *Environmental and experimental botany***,** **44**, 243-255.

Stephens W, Tyrrel S F and Tiberghien J-E (2000) Irrigating short rotation coppice with landfill leachate: constraints to productivity due to chloride. *Bioresource Technology*, **75**, 227-229.

Stolzy I and Letey (1964) Characterizing soil oxygen conditions with a platinum microelectrode. *Advances in Agronomy*, **16**, 249-279.

Stolzy l, Zentmeyer G and Roulier M (1975) Dynamics and measurement of oxygen diffusion and concentration in the root zone and other microsites. *Biology and control of soil-borne pathogens, G W Bruelh (ed) the American phytopathological society, St Paul Minn p50-54.*

Stott K G (1992) Willows in the service of man. *Proceedings of the Royal Society of Edinburgh,* **94B**, 168-182.

Stylinski C D, Oechel W C, Gamon J A, Tissue D T, Miglietta F and Raschi A (2000) effects of lifelong CO2 enrichment on carboxylation and light utilisation of *Quercus pubescens* Willd. Examined with gas exchange, biochemistry and optical techniques. *Plant Cell and Environment*, **23**, 1353-1362.

Tackett J L (1968) Theory and application of gas chromatography in soil aeration research. *Soil Science Society of American Proceedings*, **32**, 346-350.

Taiz L and Zieger E, (1998) Plant Physiology. *Sinauer Associates Inc, 2nd ED.*

Takacs Z, Tuba Z and Smirnoff N (2001) Exaggeration of desiccation stress by heavy metal pollution in *Tortula ruralis*: a pilot study. *Plant Growth Regulation,* **35,** 157-160.

Tattini M, Montagini G and Traversi M L (2002) Gas exchange, water relations and osmotic adjustment in *Phillyrea latifolia* grown at various salinity concentrations. *Tree Physiology*, **22,** 403-412.

Tezara W, Martinez D, Rengifo E and Herrara A (2003) Photosynthetic responses of tropical shrub *Lycium nodosum* to drought, soil salinity and saline spray. *Annals of Botany*, 92, 757-765.

Tezara W, Mitchell V J, Dricoll S D and Lawlor D W (1999) Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature*, **401**, 915-917.

Tiberghien J-E (1998) The effect of chloride concentration on Willow trees. *MSc Thesis, Cranfield University at Silsoe.*

U.S. department of Agriculture (1959). Diagnosis and improvement of saline and alkali soil. *Agricultural Handbook No. 60.*

Verwijst T and Wen D Z (1996) Leaf allometry of *Salix viminalis* during the first growing season. *Tree Physiology,* **16**, 655-660.

von Caemmerer S, and Farquhar G D (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta,* **153**, 376-387.

Warnaars B and Eavis B (1970) Soil physical conditions affecting seedling root growth. Mechanical impedance, aeration and moisture availability as influenced by grain size distribution and moisture content in silica sands. *Plant Soil*, **36**, 623-634.

Weih M (2001) Evidence for increased sensitivity to nutrient and water stress in a fast growing hybrid willow compared with a natural clone. *Tree physiology*, **21**, 1141-1148.

Williams M, Mahli Y, Nobre A D, Rastetter E B, Grace J and Pererra G P (1998) seasonal variation in net carbon exchange and evapotranspiration in a Brazilian rainforest: a modelling analysis. *Plant Cell and Environment,* **21,** 143-156.

Williamson R and Kriz G (1970) Response of agricultural crops to flooding depth – of water table and soil gaseous composition. *Trans ASAE*, **13,** 216-220.

Wong M H and Leung C K (1989) Landfill leachate as irrigation water for tree and vegetable crops. *Waste Management and Research,* **7,** 311-324.

WRC (2002) Landfill leachate management using short rotation coppice – Final technical report. *Eds Alker G, Gogley A and Hallett J, Repot No. CO5126.*

Xu Q and Huang B (2000) Effects of differential air and soil temperature on carbohydrate metabolism in creeping bentgrass. *Crop Science:* **40**, 1368-1374.

Yoon G L and Park J B (2001) Sensitivity of leachate and fine contents on electrical resistivity of sandy soils. *Journal of Hazardous Metals*, **B84**, 147-161.

Zang R and Wienhold B J (2002) The effect of soil moisture on mineral nitrogen, soil electrical conductivity and pH. *Nutrient Cycling in Agroecosystems,* **63**, 251-254.

Zhang S Q and Gao R (1999) Diurnal changes of gas exchange, chlorophyll fluorescence and stomatal aperture of hybrid poplar clones subjected to midday light stress. *Photosynthetica*, **36,** 559-571.

Zhang S Q, Outlaw W H and Aghoram K (2001) Relationship between changes in the guard cell abscisic acid content and other stress related physiological parameters in intact plants. *Journal of Experimental Botany,* **355,** 301-308.

Ziska L. (1998) The Influence of Root Zone Temperature on Photosynthetic Acclimation to Elevated Carbon Dioxide Concentrations. *Annals of Botany*, **81**, 717-721.

9 Appendix

9.1 Recipe for Synthetic leachate high in Chloride

Table 9.1: Recipe for synthetic leachate P.Thorn (pers. com 2001)

Chemical	*For 1 tonne*
Conc. Sulphuric acid	92.8
Conc. Propionic acid	6.1
Conc. Butyric acid	4.5
Calcium acetate	13.9
Ammonium chloride	2134.8
Sodium hydroxide	1537.2
Calcium hydroxide	285.2
Magnesium hydroxide	217.4
Potassium hydroxide	642.1
Ammonium hydrogen carbonate	890.3

9.2 Statistics for the water control PAR curves over the 2002 season

Table 9.2: Average Calculated CO_2 uptake for the water only irrigation treatment over the 4 days PAR curves were taken.

	Step							
Date of sample	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
12/04/2002	17.2	16.8	14.2	10.9	8.6	4.6	0.3	-2.3
16/05/2002	18.1	17.1	13.4	10.3	6.0	0.4	-1.8	-3.0
27/06/2002	20.2	20.0	16.1	12.1	6.6	0.4	-1.8	-2.8
25/07/2002	19.2	18.5	13.2	9.9	6.3	2.5	-0.9	-1.6
LSD	3.0	2.7	2.1	2.1	3.0	4.0	3.3	1.9
p =	0.193	0.092	0.032	0.187	0.294	0.124	0.48	0.504

9.3 Statistics for the water control CO_2 curves over the season

Table 9.3: Calculated CO_2 uptake for each of the steps for each of the dates CO_2 curves were sampled on for the water only irrigation treatment.

	Step							
Date of sample	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
21/05/2002	48.2	41.9	39.0	16.2	10.3	4.5	1.7	-2.4
26/06/2002	40.4	36.5	33.7	18.5	12.6	4.5	1.6	-2.4
29/07/2002	45.1	38.6	35.7	16.9	12.7	4.5	1.7	-0.8
LSD	22.0	16.7	18.8	6.8	6.6	1.0	0.2	3.2
p =	0.733	0.77	0.818	0.74	0.663	0.98	0.587	0.471

9.4 Conversion factor for ODR

The dimensions of the cylindrical platinum cathode were recorded with a micrometer screw gauge and ruler. The length was recorded as 8 mm and the diameter as 1 mm. Therefore the surface area was 0.025 m^2. During the measurement of ODR there are a number of values which remain constant, see Table 9.4.

Table 9.4: Constants used in the calculation of ODR from the measured current.

Constant	*Units*	*Value*
Oxygen diffusion rate to the platinum electrode (fx)	cm^{-2} min^{-1}	1920
Surface area of the cathode (A)	cm^2	0.25
Number of electrons needed for the reduction of 1 molecule of oxygen	n/a	4
Faraday's constant (F)	n/a	96500

Therefore if one incorporates the entire constants from Table 2 into Equation 6.3 we obtain a conversion value of 0.019 which can be used to interpret the diffusion rate of oxygen from measured current, see Equation 9.1.

$$\text{ODR } (g.cm^{-2}.min^{-1}) \quad = \quad 0.019.i\ (\times 10^{-8})$$

Equation 9.1: Conversion constant used for ODR calculation, where i = measured μamps.