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Procedure to control hydraulic potential variation in soil microcosms

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A b s t r a c t. In this study we designed and tested a methodology to minimize the variation of soil matric potential due to changes in soil water content, thus achieving a close control over the water regime in a microcosm, used to study the biocontrol of *Pythium sp*. infection of seeds by antagonistic bacteria. The variation of volumetric water content and matric potential were monitored at different depths during an average experimental period of 14 days in soil, contained in replicate 1 315 cm^3 microcosms, each sown with 16 sugar beet (*Beta vulgaris*) seeds and placed in a phytotron. Several experiments with target soil matric potentials of -10, -100 and -300 kPa were performed. It was found possible to maintain a matric potential of about -10 kPa in soil with minimum water content gradients between different parts of the microcosm by watering uniformly at each depth. With an appropriate watering and sampling procedure it was also possible to control ψ_m between -150 and -50 kPa, with a target value around -100 kPa; and to control ψ_m in the range between -250 and -375 kPa for a target value of -300 kPa. In all experiments spatial and temporal variability of matric potential was larger at the top than in the middle and in the bottom of microcosms. Variability in matric potential between replicate microcosms within each experiments was attributed to slight variation in packing, soil texture and drying rate, initial VWC.

K e y w o r d s: biocontrol, matric potential, microcosm, psychrometer, watering

INTRODUCTION

Plant growth and biocontrol experiments are often carried out in small soil microcosm that are assumed to be homogenous in terms of soil temperature, water content and matric potential. This assumption is rarely tested, and large gradients in temperature and matric potential can occur. Then the actual matric potential can also result in being quite different from the intended value due to hysteresis in the water characteristic.

Many experiments on interactions between seedlings and soil, or seedlings and microorganisms, that aim to achieve a uniform and controlled soil water regime are carried out in soil microcosms where matric potential is not monitored. Soil is initially wetted to a target water content, that is often chosen on the basis of a water release characteristic (Howie *et al*., 1987; Liddell and Parke, 1989; Parke *et al.,* 1986; Perreault and Whalen, 2006) and any watering is usually applied only at the surface or base of the microcosm (Liddell and Parke, 1989; Bowers and Parke, 1993). However, a variety of problems can results in large systematic errors in the actual matric potential and considerable spatial and temporal heterogeneity in the water regime within and between microcosms. These can cause differences in water availability and in nutrient supply to plants, and in activity and movement of soil microorganisms. Generally study in microcosm neglecte to monitor the water potential constantly during the trials and did not rewater with a calculated amount of water based on measurement of the previous water loss. Consequently, there is in their results an unknown uncertainty in the matric potential and by how much it may have varied before, during and after rewetting.

Movement of water in a microcosm under isothermal conditions is controlled by the hydraulic potential (ψ_h), which is the sum of matric (ψ_m) and gravitational potential (ψ_g) (Marshall *et al*., 1996). Water flows from points of high to low hydraulic potential so that, at equilibrium, the hydraulic

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potential will be the same at each point within a microcosm. In unsaturated conditions, differences in gravitational potential between different parts of a microcosm are usually small or negligible compared to differences in matric potential. Even in soil at or wetter than field capacity, (which means a fully drained wet soil, the matric potential at field capacity can vary from soil to soil) a difference in height of 10 cm corresponds approximately to a difference of 1 kPa in gravitational potential.

Whenever water is added to a microcosm, the matric potential in the vicinity will be momentarily increased to near zero and then decrease as water moves away to rewet other parts of the microcosm that have a lower potential.

Consequently rewatering from the top of a microcosm will often cause a large localized alteration in the matric potential that can take hours or even days to dissipate (Davies and Whitbread, 1989; Liu *et al*., 1997).

Watering method is likely to affect both the movement and activity of bacteria (Otten and Gilligan, 1998; Otten *et al*., 1999; Rattray *et al*., 1992). Thus it can affect the experimental results, since both are influenced by matric potential (Parke *et al*., 1986; Toyota *et al*., 1996; Turnbull *et al*., 2001; Wilson and Griffin, 1975).

Since matric potential controls the size of soil pores filled with water (Marshall *et al.*, 1996), this can have a direct effect on the ease of bacterial movement in addition to any movement caused by bacteria being carried along in the flowing water (Griffin and Quail, 1968). Furthermore, large fluctuations in potential can be caused by thermally induced vapour flow when one point of the microcosm is heated, for example, as a result of lights switching on in a phytotron.

Whilst the radiant energy selectively heats those parts of the microcosm that absorb it, the compensatory cooling of the air circulated in the phytotron cools other parts the microcosm so that is not a temperature homogenous environment. Soil temperature fluctuates during the 24 h following the light cycle simulated in a phytotron with an amplitude that depends on the position of the microcosm and the radiation and air flow environment within the phytotron.

The experiments described in this work were set up to develop a methodology to establish and maintain a uniform hydraulic potential (ψ_h) within soil microcosms wetted to target potentials of -10 kPa (field capacity), -100 and -300 kPa. The aims were to maintain a potential that was close to the target value and to minimise water flow and temporal and spatial variations of potential within microcosms. Firstly we developed and tested a procedure to water microcosms filled with soil at field capacity that would minimise gradients in hydraulic potential and hence water flow. Secondly we measured soil water potential under drier conditions to determine how uniform it was possible to maintain the hydraulic potential (ψ_h) within and between microcosms under the influence of plant water extraction and the microcosm temperature regime with two different rewatering procedures. The methodology here detailed and evaluated was then applied in several trails on bacterial motility, whose results have been already published (Schmidt *et al*., 2004).

MATERIALS AND METHODS

The need to adjust the soil at drier potential is a demanding procedure. The water release characteristic of Craibstone soil cannot be used in this case due to hysteresis, which makes quite different the relation between soil matric potential and soil water content at low matric potential, when it is calculated by drying, as it is the case of water release curve, or by wetting procedure. Then a new curve was determined by a wetting procedure, gravimetrically adjusting batches of dry Craibstone soil to a range of water content between 0.12 and 0.2 g g^{-1} (volumetric water content (VWC) – Fig. 1, gravimetric water content (GWC) – Fig. 2). After wetting, the soil was left to equilibrate for 24 h before sampling to assess the exact gravimetric water content from those very samples. A subsample of each of these was then taken to measure the water potential with the psychrometer. Before the psychrometer measurements, subsamples were left in the same constant temperature room, as the psychrometer for 48 h, in a sealed box with wetted tissue paper. A second curve was built by the same procedure used to determine the wetting curve of Craibstone soil, but after the adding of the pea mash to the soil, as an inoculum carrier for microbial trials (Fig. 2).

The microcosms used were each made of plastic cable conduit and had Perspex base plate secured by four nylon screws (Fig. 3). Each microcosm was 28.9 cm high and had a cross section of 7 x 6.5 cm (volume 1 315 cm^3). A 1 cm-thick

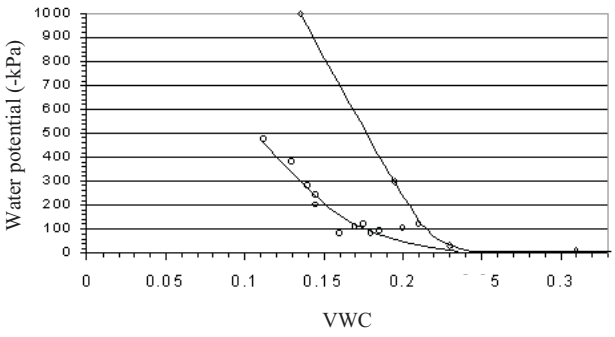

Fig. 1. Water characteristics obtained by drying or wetting Craibstone soil (VWC). The wetting curve was made by wetting dry soil (initially at 0.5% VWC) and then packing the soil to obtain a known VWC between 12 and 20% allowing the microcosm to stand for 24 h and then by sampling and measuring the water potential with a psychrometer. The drying curve (water release characteristic) was obtained by equilibrating previously saturated samples on a tension table or pressure plate at a range of matric potentials, and then determining their water content (—○— – water release curve, ○ – wetting curve).

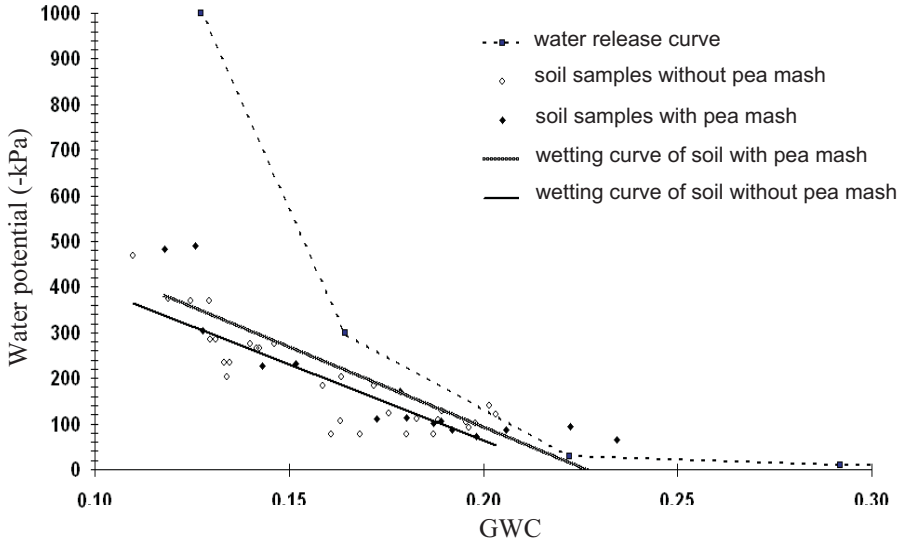

Fig. 2. Wetting and drying curves (water release) for Craibstone soil (gravimetric water content – GWC). Wetting curves include soil with and without pea mash. Wetting curves obtained by adding varying amounts of water to dry soil (0.05 g g^{-1}) soil and measuring the water potential with a psychrometer. Water release curve measured on saturated soil that had then been equilibrated on a tension table or pressure plate.

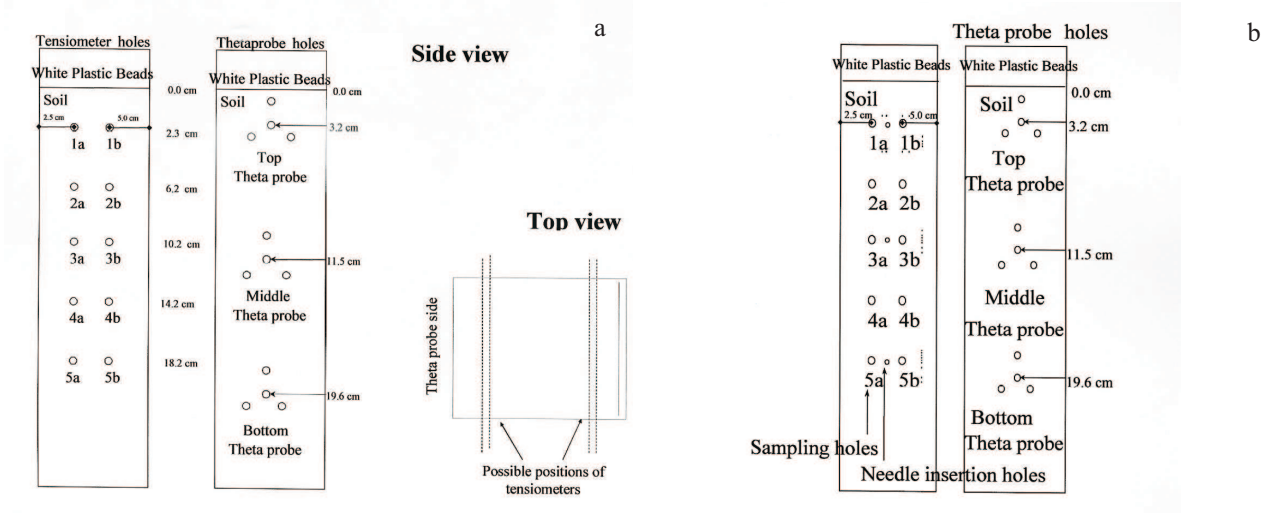

Fig. 3. Microcosm, made by a plastic cable conduit and a Perspex plate: 29.8 cm x 7 cm x 6.5 cm: a – used in experiments A, D and E; b – modified microcosm (to allow soil sampling by core) used in experiments B and C.

layer of gravel was first packed at the bottom of each microcosm to ensure aeration from the base. They were packed to a bulk density of 1 g cm^{-3} with sieved (< 3.25 mm) loamy sand that have been prewetted to one of three different water contents to give target matric potentials of -10, -100, and -300 kPa. 16 sugar beet seeds were sown at a depth of 2 cm. The soil surface was then covered with a 2 cm thick layer of white plastic beads to minimise adsorption of radiation and evaporation from the soil surface.

The microcosms were incubated in two growth chambers (phytotron SGC066.PPX.F, Sanyo Gallenka mp) with 85% relative humidity, light irradiation intensity (measured at the surface of the bead layer) of 309-400 µmol m^{-2} s^{-1}, and in 12h/12h day/night cycle until the first true leaves developed. Air temperature at night was set to 14.5°C. During daytime (based on preliminary trials) it was reduced to 11.5°C to achieve a soil temperature of 14-18°C and counteract the warming of the soil through light irradiation.

In all experiments one or more microcosms for each matric potential had theta probes (ML-1, Delta-T-Devices, Burwell Cambridge, UK) inserted at depths of 3.2, 11.5 and 19.6 cm to monitoring of volumetric soil water content (VWC) every hour using a data logger (DL3000, Delta T-Devices, Burwell Cambridge, UK).

In the soil at -10kPa target potential matric potential was measured by tensiometers (homemade, with a ceramic tube of 12 cm length and ∅ 0.4 mm fitted with a pressure sensor, SKT 600S/I; Skye Instruments. Powys, UK) positioned at 4 cm depth interval and connected to data logger Skye (Skye Instruments, Powys, UK). In the drier soils (-100 and -300 kPa) water potential was monitored by sampling replicated microcosms and measuring water potential of these samples by a thermocouple psychrometer (SC-10 Decagon Devices, Pullman, WA, USA). The water potential was assumed to be equal to matric potential since the osmotic potential of the soil was negligible.

In experiment (A), with a soil target potential of -10 kPa, different watering procedures were compared. Four experiments (B, C, D, E) with drier soil with and without pea mash were carried out with two different sampling methodology and watering procedures (Table 1).

Average hourly drying rate was measured on four microcosms filled with soil at field capacity, during 14 days of incubation in both of the chambers in different positions. The positions of the microcosms were randomised during the experiments after watering to compensate for differences in drying rate in different part of the growing chamber. All the monitoring of matric potential was done in function of the volumetric water content because this is the parameter measured by the theta probes.

-10 kPa regime (experiment A)

The soil was wetted to water content of 0.32 m^3 m^{-3} to obtain a target matric potential of -10 kPa. This water content was based on a previously determined soil release curve. The corresponding gravimetric water content of the soil was checked before the packing gravimetrically.

Each tensiometer connected to its own pressure sensor was calibrated by immersing in a water beaker which was displaced at different heights from that where it was located the pressure sensor thus generating a series of negative water pressures. Then the tensiometers were inserted at seed depth (2.3 cm) and at 6.2 cm depth (chosen to be near the low limit of most root growth during each run) into microcosms before they were packed. After packing each tensiometer was filled (using a syringe) with de-aired water, leaving a small filled space (*ca*. 1 ml) to allow for temperature fluctuations. A plastic tube was attached at either end of each ceramic rod. At one end, this was clamped after the tensiometers had been filled. The other end was connected to the pressure sensor (Fig. 4).

Each theta probe, which has an output in mV, was calibrated by inserting it in water, air, air-dry and moist (0.25 g g^{-1}, water content) soil. In each case, the probe was first inserted through a plastic sheet, made from the wall of the microcosm, to compensate for any wall-effect. The average of five independent readings was used to construct calibration curves for each probe at each water content.

Two watering methods ('a' and 'b') were tested in microcosms fitted with two tensiometers and three theta probes. Both methods were tried as possible improvement to the common method of watering at the surface, which could drag the bacteria downwards. Rewatering was carried out each time tensiometers recorded a matric potential lower than -15 kPa. Water was added from a hypodermic needle to each microcosm in measured amount by inserting the needle 3-4 mm though a suba seal at the five depths shown in Fig. 3 in positions not occupied by tensiometers. At each depth water was added to either side of the microcosm. In 'a' method or 'uneven' the watering was concentrated where theta probes recorded the greatest water loss and was proportional to the theta probes readings at each depth. In 'b' method or 'even' an equal amount of water was added at each depth, based on the average water loss from the microcosm calculated as average of the theta probe readings at each depth. In both methods, 20% more water was added than indicated by the theta probes to take account of hysteresis.

-100 and -300 kPa regime(Experiments B, C, D, E)

Four experiments (B-E) were carried out to establish a reliable methodology to control soil matric potential at target values of -100 and -300 kPa (Table 1). The matric potential was monitored as water potential (assumed to be equal to soil matric potential, since the osmotic potential was considered negligible) on samples from replicate microcosms with a Richards psychrometer (SC-10 Decagon Devices, Pullman, WA, USA). This instrument was preferred to alternative methods accounting the ranges of soil water potential and relative humidity involved (Agus and Schanz, 2005; Bakker *et al.*, 2007; Skierucha, 2005). In each experiment a control microcosm bearing three theta probes was set up to measure the VWC at 3.2, 11.5 and 19.6 cm depth, except that there was no probe at 19.6 cm in experiment E. Watering was done uniformerly. Theta probe results were converted to VWC using the manufacture's calibration (ML1 Theta Probe User Manual, Burwell Cambridge, UK).

Different sampling procedures and times were adopted in each experiment to determine the best way to collect soil samples with matric potential,that best represented the water regimes inside the microcosms during the runs.

In experiment B and C the soil was sampled from the microcosm by a core (∅ 0.4 mm) after removing a suba seal and was replaced with more soil adjusted to be at about the same matric potential and kept in the phytotron under the same conditions. After subsampling, the subsamples were stored in the psychrometer room for 24-48 h and then measured.

The sampling was carried out in B experiment after each watering at different intervals (6,12, 24, and 48 h after the first watering and 18, 24, 48 and 72 h after the second one).

T a b l e 1. Sampling and watering of five experiments

Exp.	Soil Ψ_m (-kPa)	Pea mash	Ψ_m measured by	Sampling time	Sampling depths (cm)	How sampled	Sampling time based on	Number of samples at each depth	Distribution of rewatering	Amount of rewatering based on
Number of microcosms used										
2 (1 for each water distribution)										
A	10	–	tensiometerat depth 2.3 and 6.2 cm	n.a.	n.a.	n.a.	tensiometer	n.a.	Even or uneven	theta probes
16 (2 for each sampling time)										
B	300	–	psychrometer	6, 12, 24, 48 h after the 1st watering 18, 24, 48, 72 h after the 2nd watering	3.2, 11.5, 19.6	core	theta probe	1	Uneven	theta probes
12 (6 for each Ψ_m, 2 for each sampling time)										
C	100300	++	psychrometer"	24 and 96 h after packing and at the end	3.2, 11.5, 19.6	core and spatula (*)	theta probe	1	Uneven	theta probes
18 (9 for each Ψ_m, 3 for each sampling time)										
D	100300	– –	psychrometer"	36 h after packing and 36 h after 1st and 2nd watering	3.2, 11.5	core and spatula (*)	microcosm weighing	4	Uneven	weighing
24 (12 for each Ψ_m, 3 for each sampling time)										
E	100300	++	psychrometer"	1 and 36 h after packing, 48 after watering and at the end	3.2, 11.5	core and spatula (*)	microcosm weighing	1	Uneven	weighing

n.a. – not applicable, *microcosm was open and sampled at each depth with a spatula.

In the C experiment, the soil was sampled before and after the packing to detect the possible loss of water during the packing. At end the experiment an additional sampling was done destructively by collecting the sample after opening a further set of microcosms. The soil was collected at three depths (3.2 , 11.5 and 19.6 cm).

In experiments D and E (D without pea mash and E with pea mash) the subsampling procedure was modified as follows to reduce any water loss due to drying during the sampling and to avoid the disturbance caused by the core. The microcosms were weighed in the phytotron room, sealed in a plastic bag with a moist paper towel to keep the air moist, and then moved to the room where the psychrometer was located. There, after 48 h, 4 samples at the four corners (a, b, c, d,) of the microcosms of D experiment were collected with a spatula at 3.2 and 11.5 cm depth. In experiment E only one sample was collected at 3.2 and 11.5 cm depth. This was a destructive procedure after which there was no further use of the microcosm.

In D the soil was sampled 18 h after packing and 36 h after each watering event, in experiment E 1 and 36 h after packing, 48 h after each watering, and at the end of the experiment.

To maintain the matric potential during the incubation period, columns were rewatered at different heights through suba seals with a syringe inserted for 1 cm into the microcosm. In all experiments (B-E) 20% more water was added than calculated by change in theta probe readings in the control microcosms or by weight in the other ones to compensate for the effect of hysteresis, generated by multiply wetting and drying cycles within the microcosm. It was assumed from drying and wetting curve (Fig. 2) that an over amount would assure a fast reestablishment of the desired matric potential. Rewatering was done in experiments (B and C) when the water content fell by more than 0.01 m^3 m^{-3} below the target value in the -100 and -300 kPa soil. The amounts of water added were calculated using the theta probe readings, giving more water in the region where the soil was drying quicker. This method assumes that the water loss from the control microcosm did not differ significantly from that from the other microcosms.

Standard error was calculated for all the measurements. When the water potential measures were not normal distributed their log transformed values were used instead. One way ANOVA with replicate was carried out on data from B to E experiment to evaluate the effect of depth; two way ANOVA without replicate was carried out on data from experiment D to evaluate the effect of the sampling position and the depth. The significance of difference in drying rate in soil with and without pea mash was tested by t test.

RESULTS AND DISCUSSION

-10 kPa regime

Matric suction at 2.3 and 6.2 cm, and temperature at 2 cm below the soil surface were monitored in two microcosms kept under our standard phytotron conditions. The microcosms were watered when the matric suctions had increased to 17.5 and 15 kPa, respectively. In the microcosm 8 ml of water was added, 4ml each at the depths of 10.2 and at 14.2 cm, where decreases in water content were observed (Fig. 5a). 30 ml of water was uniformly distributed down the microcosm by adding 6 ml of water at 2.3, 6.2, 10.2, 14.2 and 18.2 cm depth (Fig. 5b).

The tensiometer in microcosm Fig 3a responded more slowly than in microcosm C to daily fluctuations in potential, due to a larger volume of air inside the tensiometer. Nevertheless, the delay in tensiometer response of about 24 h at 6.2 cm must correspond approximately to the time required for the rewetting front to rise from the 10.2 depth at which water was added. In contrast, in microcosm C, both tensiometers responded within an hour of rewatering. Consequently, the more uniform addition of water reduced the time required for the hydraulic potential to approach equilibrium within the microcosm. This also implies that there were smaller gradients in potential between different parts of the microcosm during the 24 h after rewatering.

Figure 3b shows typical results for matric suction and temperature that were observed when tensiometers had a fast response time and when water was added uniformly during rewatering. The daily fluctuations in matric suction cannot initially be due to plant water extraction since seedlings did not emerge until day 7. tensiometer sensitivity was also insensitive to temperature fluctuations, consequently, the fluctuations must be due to the thermal effects (probably thermally driven vapour flow) caused by the differences in temperature that must have existed between the top and bottom, and outside and centre of the microcosm just after the lights had switched on or off. For example, Fig. 5b shows that, even when the air temperature in the phytotron was reduced by about 3°C, to compensate for the heating of the outside parts of the microcosm that were exposed to radiation, there was still a 2°C rise in soil temperature near the top of the microcosm. As shown by the vertical gradients in hydraulic potential, this produced a tendency for downward water flow after the lights had been switched on and upward flow after they had been switched off. However, these gradients were comparatively small and did not result in such a large change in potential as that due to rewatering. The daily temperature (and consequently matric suction) fluctuations were reduced after rewatering. This was not a consequence of watering but is because the positions of microcosms within

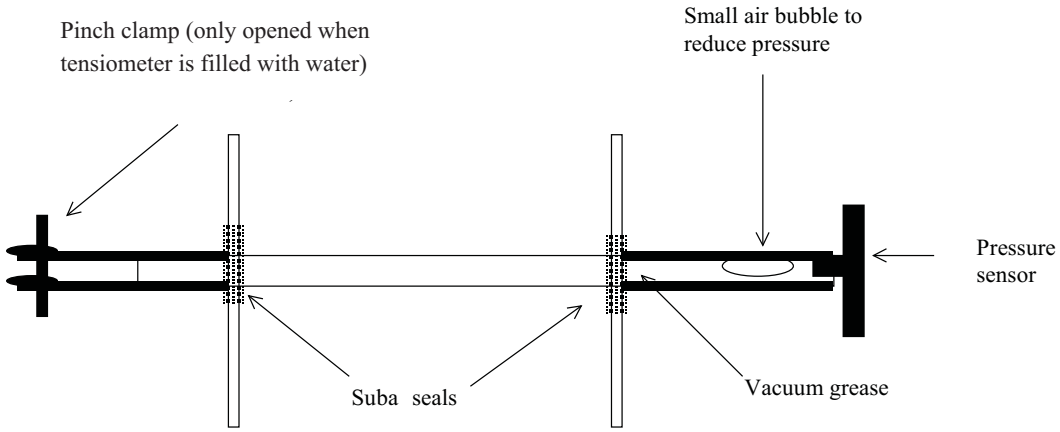

Fig. 4. Home made tensiometers, inserted in the microcosm and filled with de-aired water.

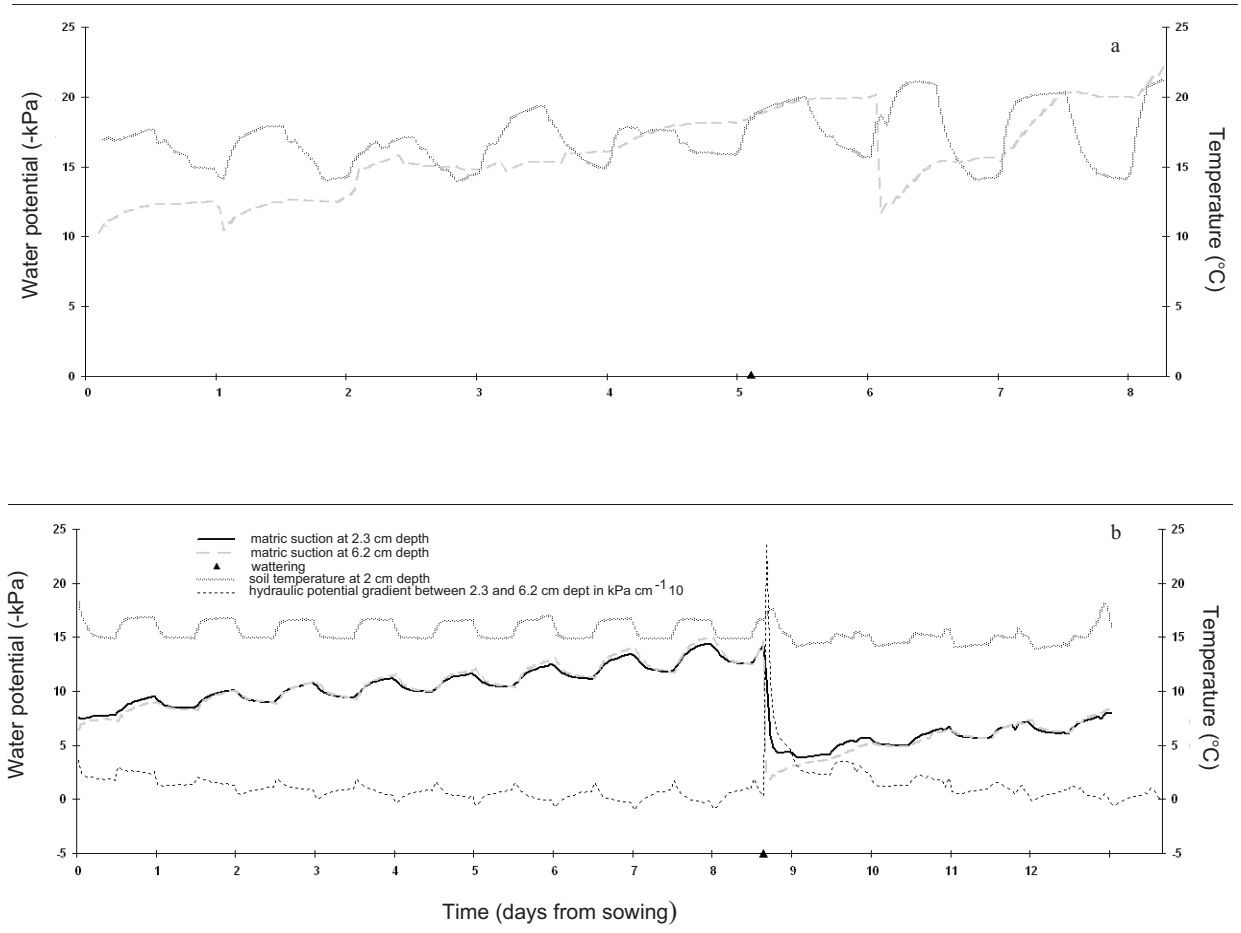

Fig. 5. Data from experiment A, matric suction, soil temperature and hydraulic potential gradient, versus time in two microcosms filled with soil adjusted at -10 kPa, two different watering procedures applied, (run in a growth chamber with 85% relative humidity, 12h/12h day night cycle, soil temperature set to 14 -18°C); a – no uniform watering (uneven), water injected through suba seals at each depth of the microcosm was equal to 1.2 times water loss recorded by the theta probe at such depth, the tensiometer at 6.2 cm depth was not working; b – uniform watering (even), the amount of water injected at all depths was calculated as 1.2 times the average of the water loss recorded by the theta probes at each depths, the gradient is calculated as [ΔkPa/ (6.2-2.0)].

the phytotron were rerandomised after rewatering. In many experiments (results not shown), we found that the magnitude of surface soil temperature fluctuations varied according to the position of the microcosm within the phytotron (although we have observed that this effect is much smaller in a more recent, electronically controlled version of this phytotron).

-100 and -300 kPa regime

From the wetting curves (Fig. 2) the target values of GWC for -100 and - 300 kPa were identified as 0.13 and 0.2 g g^{-1}, respectively. Adding 2.75% pea mash to the soil did not substantially change the wetting curve (2.75 % of pea mash adds about 1% mass of water to the soil). The scattering of the points about the wetting curves was probably mainly due to the variability of the packing inside each sample cups of the psychrometer, since both soil disturbance and the degree of packing can influence the matric potential. A comparison between the drying (water release) and wetting curves for Craibstone soil clearly demonstrates a large hysteresis effect.

The results of the -300 kPa in experiments B and C (Fig. 6a, b) show that, after packing, the VWCs at different depths differed only slightly. However, VWCs measures with the theta probes were not always as close as expected to the target values which may indicate limitations in the wetting procedure that was used. The water potentials in both the experiments were extremely variable after the watering. There were no obvious trends of water potential with depth and the water potentials did not vary consistently with the water contents of the same subsamples. The largest variation between replicates (error bars) is shown by samples collected immediately after the watering.

The water content of the upper part of the soil (3.2 cm depth) with and without pea mash declined more quickly after watering the at the others depths.. In fact samples at

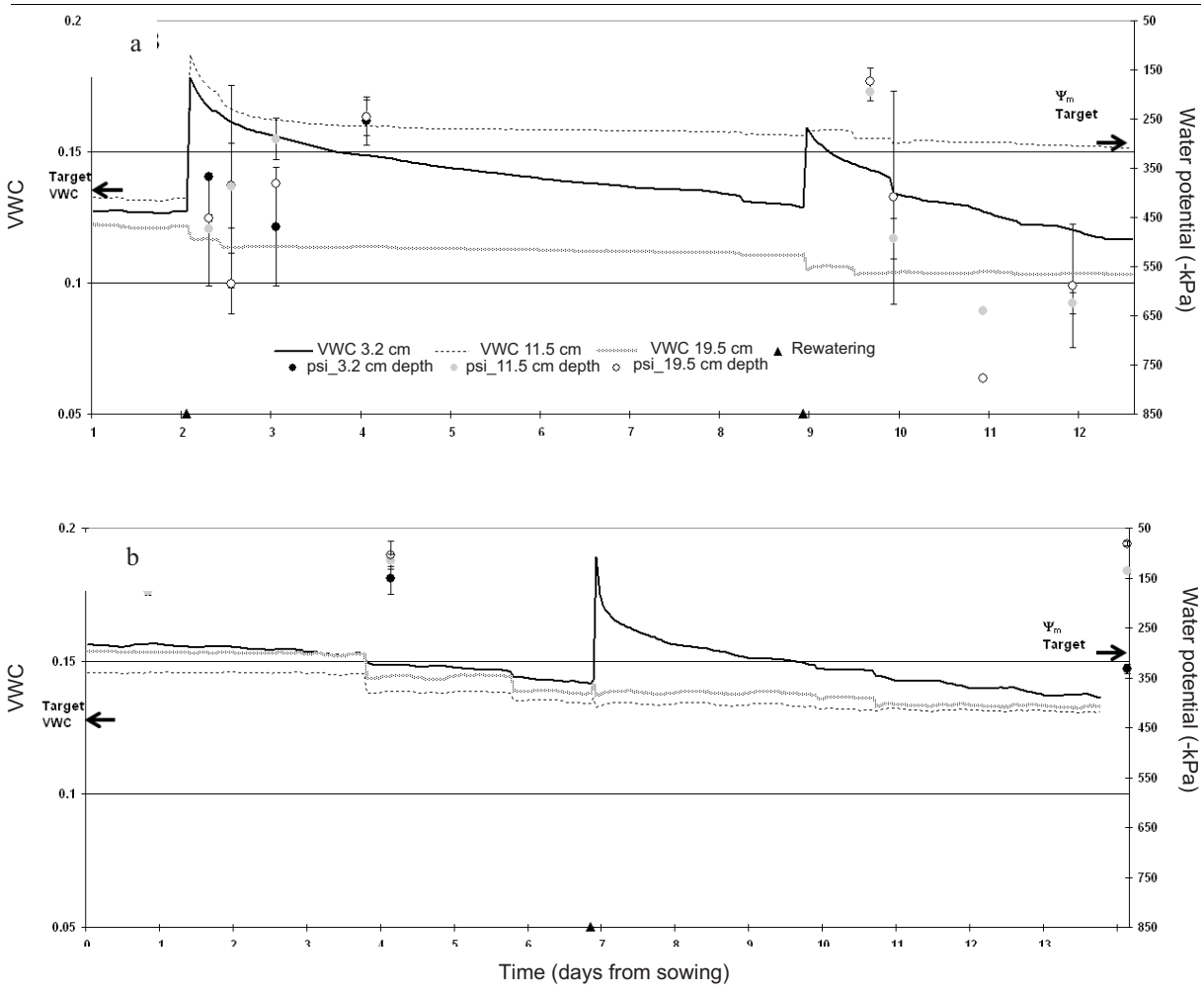

Fig. 6. Volumetric water content and water potential (psi) *vs*. time at three depths sampled by core from two replicate microcosms at each event, a – experiment B, b – experiment C (each point represents mean of two replicates and the bars are ± one standard errors).

3.2 cm depth after the second watering in Fig. 5a were lower than the calibration range of the psychrometer (data not included in Fig. 6a). This effect may be due to hysteresis, indicating that more water should have been added at this depth. Otherwise the water potentials were within a factor of two of the target value *ie* -150 to -600 kPa.

Core sampling directly from microcosms compressed the soil, and the subsequent packing of soil in the psychrometer cups could both have altered the water potential of the subsamples by altering their pore size distribution and introducing minor hysteresis effects.

Figure 7a, b compare water potentials at different depth from samples of the same microcosms at end of C experiment, collected by coring or with a spatula after the microcosms were opened. There was a tendency for the replicated spatula samples to be less variable than core samples and they also showed less variation with depth in the -300 kPa soil, although this may be just a chance occurrence (Fig. 7c, d).

Figure 8a, b show results from soil adjusted to -100 and -300 kPa in experiment D. This again shows a considerable variability of potential among replicate microcosms. No statistically significant differences were found between depths and between sampling positions within the same microcosm sampled at four corners at each depth. A comparison between Figs 6-8 shows that the new sampling procedure reduced the variability among replicate microcosms and the soil was maintained around the target values of -100 and -300 kPa to within a factor of less of two times.

Thanks to the smaller errors bars for water potentials in Fig. 8a, b, it is clear that the upper layer (at 3.2 cm) was always drier, both before and after watering, that soil at 11.5 cm depth. In this experiment VWC values correlated well with water potential. The method of watering based on weighing every microcosm and then adding 1.2 times the weight loss but distributed according to changes in theta probes readings, gave a much better control of the water potential.

In experiment E, 2.75% of pea mash added into the soil in the top 9 cm, formed several clumps of soil and pea mash in the upper part of the microcosm. This also occurred in experiment C but it did not effect the relationship between VWC and the water potential.

In the experiment E (Fig. 9a, b) the VWC and the water potential values indicate that top region (3.2 cm depth) was drying much faster than at 11.5 cm. However, in the similar experiment D (Fig. 8a, b) in which no pea mash was present, faster drying at 3.2 cm was not really so apparent. The experiment E shows two differences between soil with and without pea mash (experiment D). Firstly the pea mash resulted in faster drying near to the surface. However, the addition of pea mash decreased the water loss from the

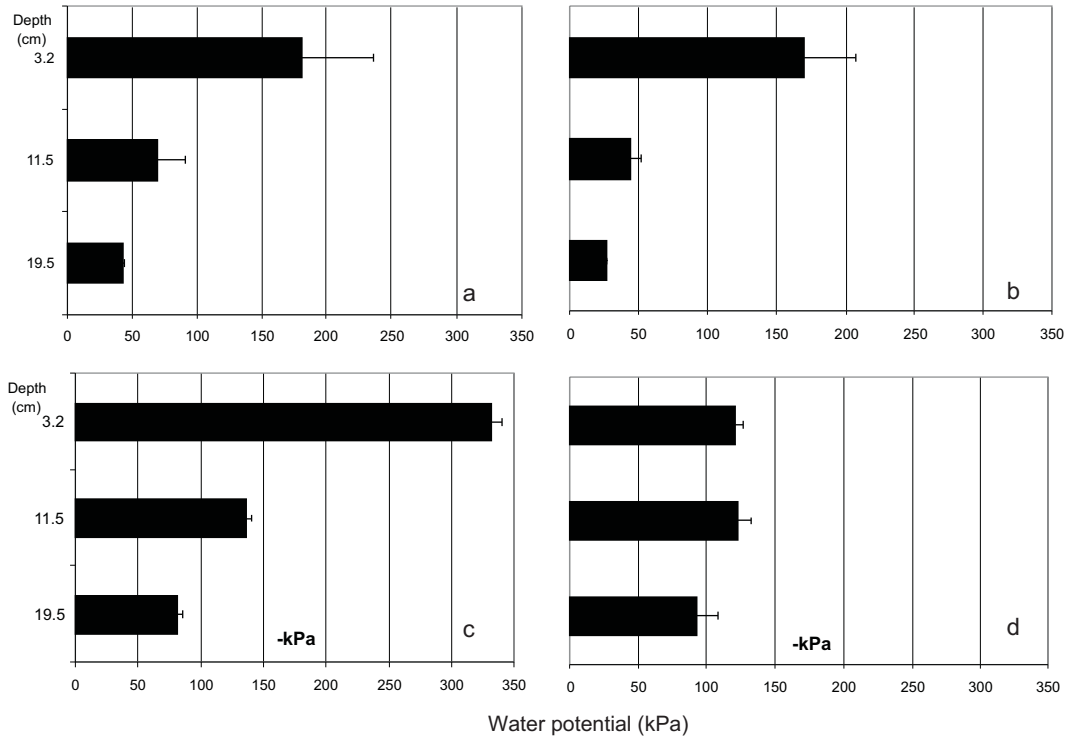

Fig. 7. Experiment C. Matric potential of samples collected at the end of the 14 day run by two different methods at three depths from two replicate microcosms. Each point represents mean of two replicates and the bars are one standard error; a – -100 kPa soil with pea mash, sampling carried out by coring; b – -100 kPa soil with pea mash, sampling with a spatula after having opened the microcosm; c – -300 kPa soil with pea mash, sampling carried out by a coring; d – -300 kPa soil with pea mash, sampling with a spatula after having opened the microcosm.

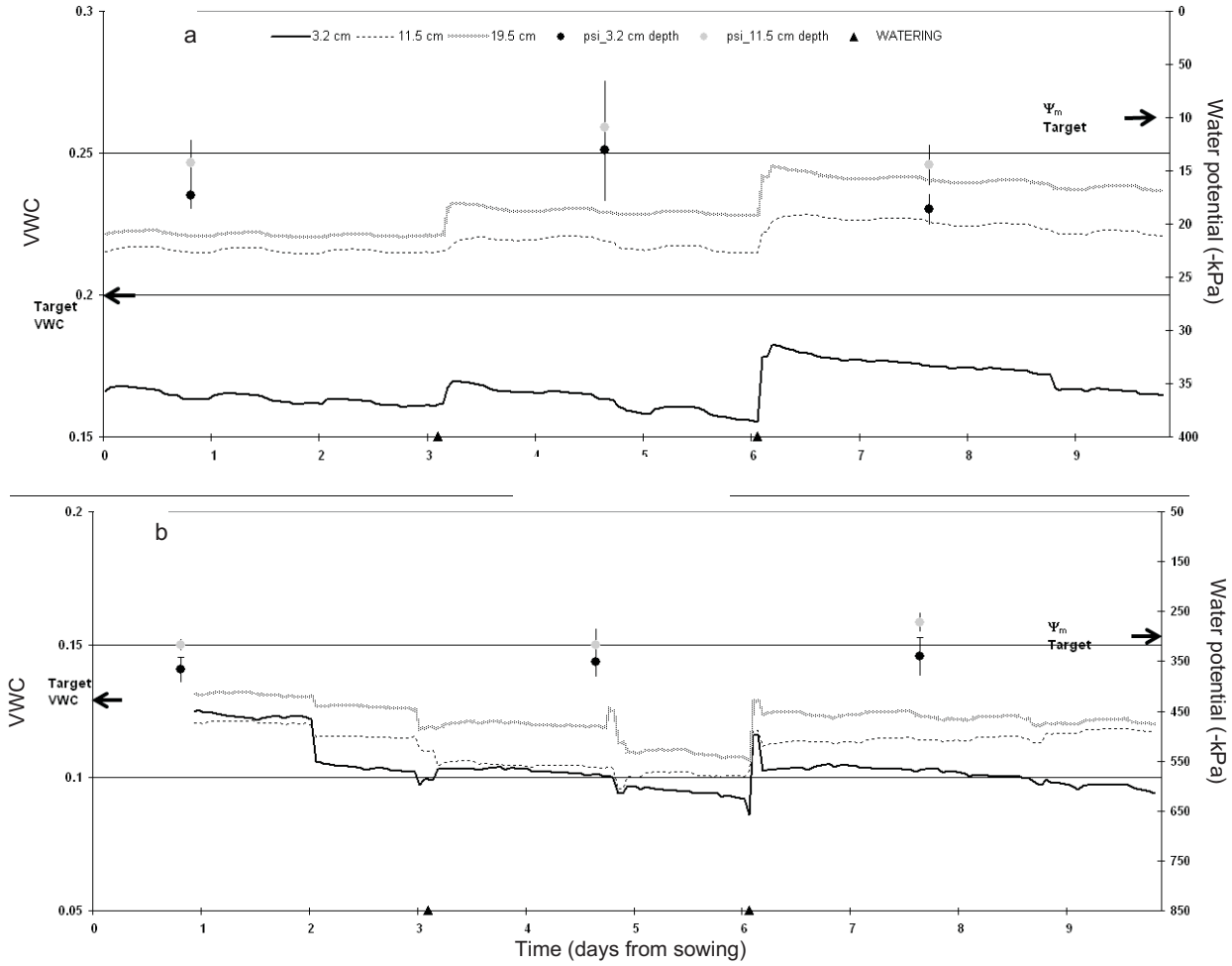

Fig. 8. Experiment D. Volumetric water content and water potential (psi) *vs*. time, sampling destructively at two depths: a – 3.2 cm, and b – 11.5 cm (from three replicate microcosms at each event, each point represents mean of 12 replicates – 4 replicated samples at each depth for three microcosms). Bars are ± standard errors.

microcosms and only one watering was required after 5 days in both the soil matric potential regimes. Average daily water loss from microcosms with pea mash was 0.66±0.4 g, which were significantly less ($P < 0.01$) than that from those without (1.15±0.6 g).

These differences were likely to be a result of the pea mash addition, which changed the relation between VWC and matric potential in the top layer. Pulling together the water potential and VWC values determined on all samples before and after watering (Fig. 10a) shows that was no significant difference in the water characteristic curve of soil with out pea mash determined before and after rewatering whereas soil with pea mash (Fig. 10b) shows a significant shift in the water characteristic. This implies that the physical properties of the soil with pea mash altered during the course of the experiment.

Figure 11 shows a further clear difference between the behaviour of soil with and without pea mash. This figure shows the gradient in hydraulic potential (expressed as the difference between potentials at 3.2 and 11.5 cm depth) versus the potential recorded at 11.5 depth. In most cases there is a clear tendency for a greater potential to exist within the soil with pea mash. This implies that it had a lower unsaturated hydraulic conductivity, consistent both with its greater observed degree of aggregation, a slower overall rate of drying by surface evaporation but also with a further rate of drying for soil near to the surface. The implication of these results is that highly aggregated soil, which has a low unsaturated conductivity at these potentials, may be especially difficult to maintain at constant water potential.

In soil drying from saturation to air-dryness the matric potential decreases with the decreasing of soil water content. However this relation curve changes in soil re-wetting. If the process is reversed at any stage of wetting or drying between saturation and air-dryness, at the same matric potential the drying soil has water content higher than the wetting soil. This means that at the same water content value the matric potential in wetting soil is higher than in drying soil. This

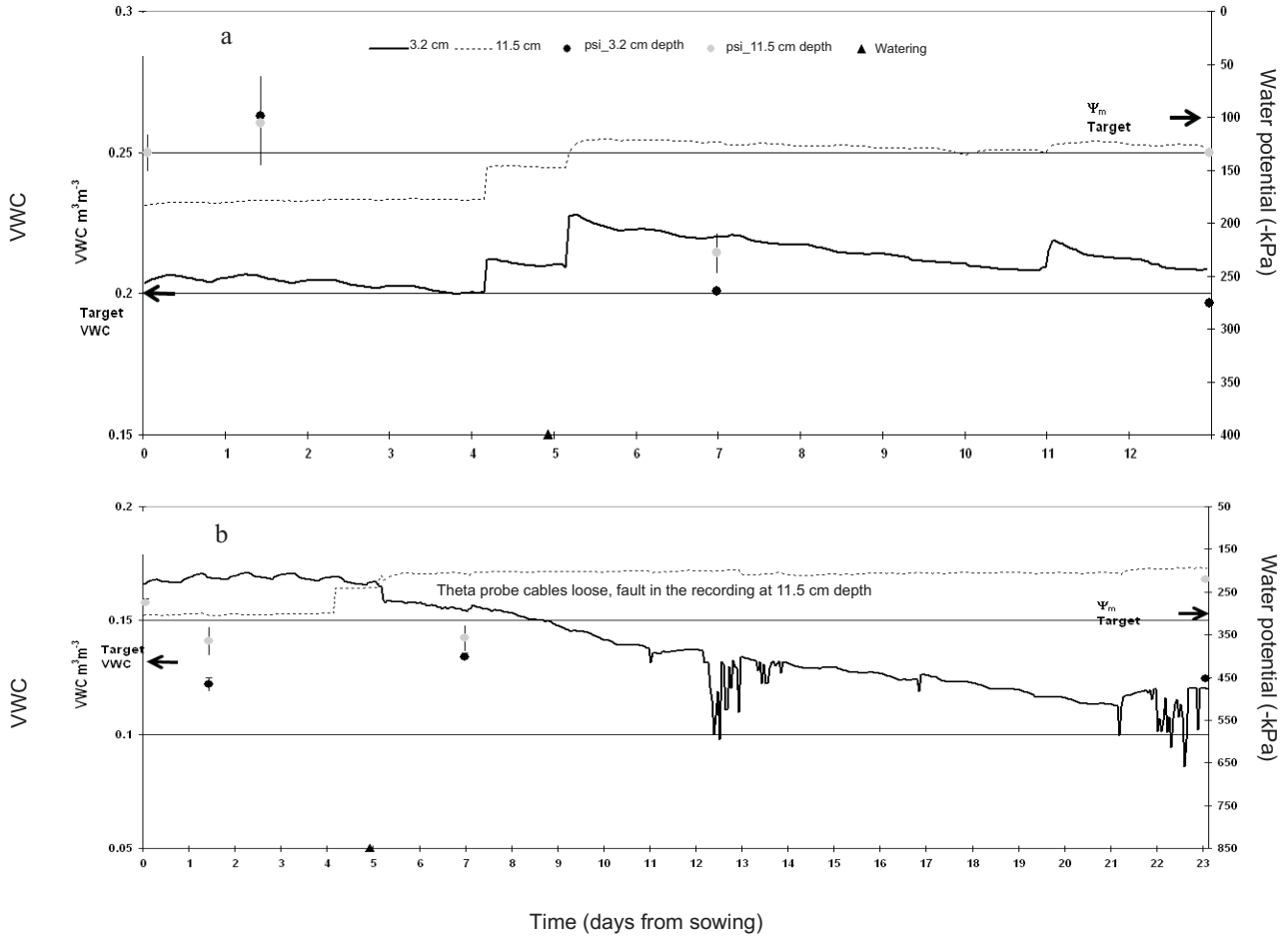

Fig. 9. Experiment E. Volumetric water content and water potential (psi) *vs*. time sampled destructively at two depths: a – 3.2 cm, and b – 11.5 cm (from three replicate microcosms at each event, each point represents the mean of three replicates). Bars are ± standard errors.

hysteresis is due to the different contact angle between water and pore walls in drying and wetting soils. In Craibstone soil hysteresis effects can introduce systematic errors in the estimation of matric potential of up to 3 times or more (Fig. 1). For example, for a target potential of -300 kPa rewetting the soil to a water content based on its water release curve would actually have brought the soil to about -100 kPa. Since sveral authors (Howie *et al*., 1987; Liddell and Parke, 1989, Parke *et al.*, 1986; Perreault and Whalen, 2006) working on microbial and plant trials in microcosms obtained their target matric potentials by wetting samples to a target water content that was obtained from a water release characteristic, most previous workers have systematically underestimated the true matric suction *ie* overestimated the potential of their soil by a factor that may be three or more times.

Rewatering microcosms from the top generate a percolation which will cause large and uncontrolled modification of the matric potential due to the creation of single or multiple (when preferential water pathways are also present) water fronts within the microcosm. Thus redistribution of water and equilibration of matric potential can be hardly achieved leaving some zones in a much wetter state. The rewatering at depth tested in the present work was found to delay rewetting (over a 4 cm depth) by about one day and would cause perturbations in water regime. In fact the accurate control of soil water content and matric potential, which are required to minimise their variability both between and within microcosms resulted more difficult to be achieved than has previously been assumed.

The combined use of theta probes and tensiometers was found to be a suitable method for monitoring microcosms at field capacity condition. The use of a psychrometer to measure lower matric potentials was also sufficiently accurate when the sampling was done destructively from replicate microcosms, after having equilibrate their soil temperature with that of the instrument. At a soil matric potential of -10 kPa, rewatering microcosms by adding equal amount of water at three depths was found to minimise the vertical gradient in

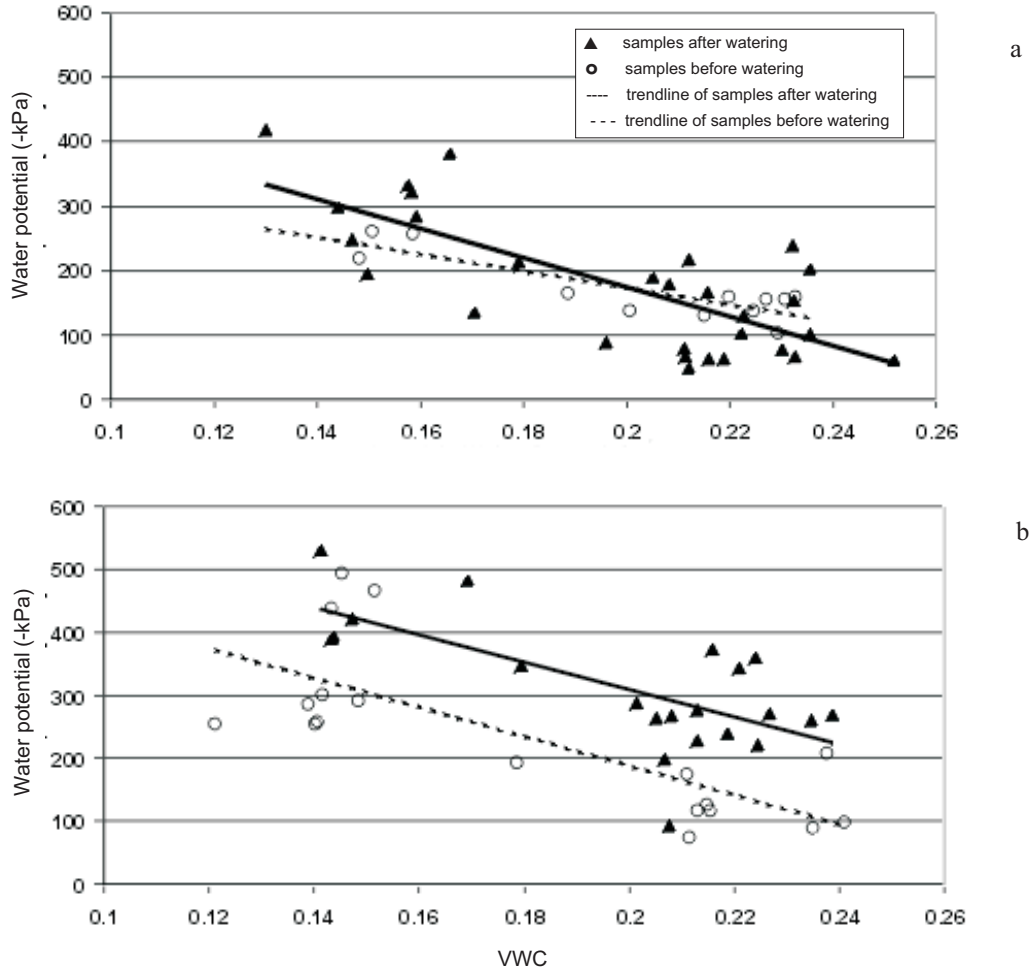

Fig. 10. Water potential *vs.* volumetric water content in soil samples from experiments B, C, D, and E, the data are organised in samples collected before or after watering the microcosms: a – soil without pea mash, b – soil with pea mash.

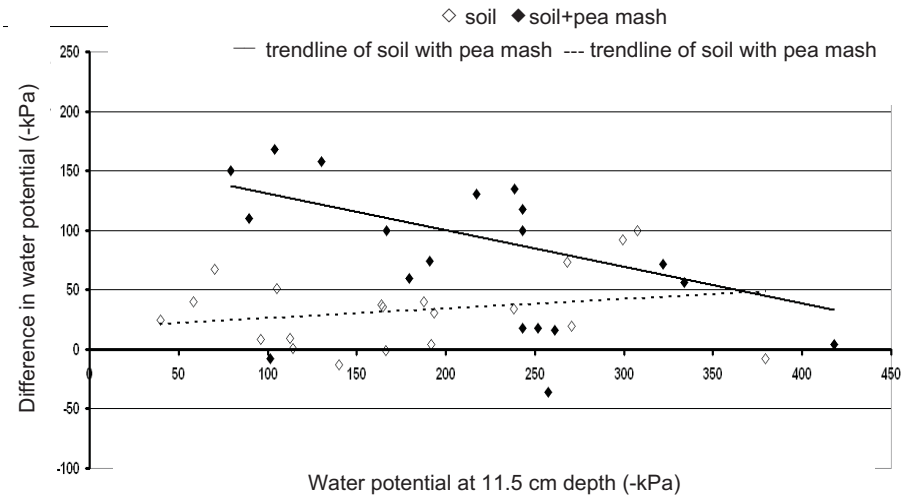

Fig. 11. Difference of water potentials between soil samples collected at 3.2 and 11.5 cm depths *vs.* water potential of soil samples collected at 11.5 cm depth from experiments B, C, D and E.

hydraulic potential. This will minimise also the amount of water flow between different depths after rewatering.

At target potentials of -100 and -300 kPa the same system of rewatering kept the water potential to within 50% of the target values, during 12-14 days. Within any given microcosm water potential varied significantly with depth, but not with position within the microcosm at any given depth. However, there was also a considerable variation in potential between different replicate microcosms, which we attribute to other factors such as: microcosm packing, differences in initial VWC and in evapotranspiration rate. Consequently, although there were only small differences in volumetric water content among replicate microcosms there could be still large differences in water potential. In conclusion the proposed procedure was able to minimise matric potential variations at the same depth and only within the same microcosm.

An additional source of variability was the (pea mash) substrate used to inoculate the soil with pathogens, which changed the shape of the water release curves after rewetting and altered soil aggregation and the rate of water loss from the microcosms.

CONCLUSION

1. A better control of the matric\water potential in microcosms used in soil microbial and plant studies could be achieved by:

– a more uniform temperature and radiation regime within the phytotron,
– a better design of microcosm, in which soil does not need to be packed in layers *ie* soil disposed as thin layer between 2 plastic sheets,
– a pathogen inoculation procedures which does not require the addition of a substrate to the soil.

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