The effect of drought and interspecific interactions on the depth of water uptake in deep- and shallow-rooting grassland species as determined by $\delta^{18}O$ natural abundance.

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Abstract

Increased incidence of weather drought, as predicted under climate change, has the potential to negatively affect grassland production. Compared to monocultures, vertical belowground niche complementarity between shallow- and deep-rooting species may be an important mechanism resulting in higher yields and higher resistance to drought in grassland mixtures. However, very little is known about the belowground responses in grassland systems and increased insight into these processes may yield important information both to predict the effect of future climate change and better design agricultural systems to cope with this.

This study assessed the effect of a 10-week experimental summer drought on the depth of water uptake of two shallow-rooting species (*Lolium perenne* L and *Trifolium repens* L) and two deep-rooting species (*Chicorium intybus* L and *Trifolium pratense* L) in grassland monocultures and four-species-mixtures by using the natural abundance $\delta^{18}O$ isotope method. We tested the following hypotheses: 1) drought results in a shift of water uptake to deeper soil layers, 2) deep-rooting species take up a higher proportion of water from deeper soil layers relative to shallow-rooting species, 3) as a result of interspecific interactions in mixtures, the water uptake of shallow-rooting species become shallower when grown together with deep-rooting species and vice versa, resulting in reduced niche overlap.

The natural abundance $\delta^{18}O$ technique provided novel insights into the depth of water uptake of deep- and shallow- rooting grassland species and revealed large shifts in depth of water uptake in response to drought and interspecific interactions.

Compared to control conditions, drought reduced the proportional water uptake from 0-10 cm soil depth ($PCWU_{0\text{-}10}$) of *L perenne, T repens* and *C intybus* in monocultures by on average 54 %. In contrast, the $PCWU_{0\text{-}10}$ of *T pratense* in monoculture increased by 44 %, and only when grown in mixture did the $PCWU_{0\text{-}10}$ of *T pratense* decrease under drought conditions. In line with hypothesis 2, in monoculture, the $PCWU_{0\text{-}10}$ of shallow-rooting species *L perenne* and *T repens* was 0.53 averaged over the two drought treatments, compared to 0.16 for the deep-rooting *C intybus*. Surprisingly, in monoculture, water uptake by *T pratense* was shallower than for the shallow-rooting species ($PCWU_{0\text{-}10} = 0.68$).

Interspecific interactions in mixtures resulted in a shift in the depth of water uptake by the different species. As hypothesised, the shallow-rooting species *L*

perenne and *T repens* tended to become shallower, and the deep-rooting *T pratense* made a dramatic shift to deeper soil layers (reduction in $PCWU_{0-10}$ of 58 % on average) in mixture compared to monoculture. However, these shifts did not result in a reduction in the proportional similarity of the proportional water uptake from different soil depth intervals (niche overlap) in mixtures compared to monocultures.

There was no clear link between interspecific differences in depth of water uptake and the reduction of biomass production under drought compared to control conditions (drought resistance). *Chicorium intybus*, the species with water uptake from the deepest soil layers was one of the species most affected by drought. Interestingly, *T pratense*, which was least affected by drought, also had the greatest plasticity in depth of water uptake. This suggests that there may be an indirect effect of rooting depth on drought resistance, as it determines the potential plasticity in the depth of water uptake.

Introduction

Both the frequency and the intensity of extreme weather events is predicted to increase under climate change (IPCC, 2013) . Climate models predict that the climate in Central Europe will be characterised by increasing temperatures, reduced summer precipitation and increased frequency of extreme events (Christensen, 2003;Schär, 2004). These discrete events include droughts, heat-waves and storms, and can have a large impact on a variety of ecosystem functions and services (Lehner et al., 2006). Increased incidence of drought has the potential to disrupt crop and grassland production, and there is a need to consider adaptation options to support global food security. Research on temperate grasslands shows a strong negative effect of drought on aboveground production (Gilgen and Buchmann, 2009;Grime et al., 2000;Kahmen et al., 2005;Vogel et al., 2012;De Boeck et al., 2008), however, there is high variability in the observed responses. This variability could be related to differences across experiments in the severity (Vicca et al., 2012) and timing of the drought stress, as well as differences in plant functional types present in the ecosystem. In the current study, we focus on studying different plant functional types, i.e. deep-rooting and shallow-rooting grassland species.

It is often assumed that plants respond to water shortage in the (upper part of the) soil by shifting water extraction to deeper soil layers that generally have higher levels of water (Sharp and Davies, 1985;Garwood and Sinclair, 1979). By doing so, plants are able to delay loss of turgor, prevent stomatal closure, and maintain a high rate of photosynthesis. Rather than by a complete adjustment of the root density profile, plants can adapt to drought by rapidly developing fine roots (Coelho and Or, 1999), or by increasing the activity and efficiency of deep roots (Sharp and Davies, 1985;Kulmatiski and Beard, 2013). More deeply-rooted plants are more likely to survive extended periods of drought by accessing lower soil layers that contain higher soil moisture levels (Chaves et al., 2003). However, there are quite varied responses in the few studies of grassland plants that have investigated the effect of drought on rooting depth (Skinner, 2008;Garwood and Sinclair, 1979;Jupp and Newman, 1987), or on the depth of water uptake (Asbjornsen et al., 2008;Kulmatiski and Beard, 2013;Nippert and Knapp, 2007b;Nippert and Knapp, 2007a;Grieu et al., 2001) and only very few studies were conducted in temperate grassland systems with grasses and herbaceous plants only (Grieu et al., 2001;Prechsl, 2013).

Plant species diversity in both semi-natural (e.g. Tilman et al., 1996;Hector et al., 1999;Kennedy et al., 2002;Tilman et al., 2002) and agricultural (e.g. Finn et al., 2013;Kirwan et al., 2007;Nyfeler et al., 2009;Nyfeler et al., 2011) ecosystems has been related to higher primary production, nutrient retention, resistance to weed invasion and stability in response to disturbance, and is often attributed to complementarity in a variety of plant traits and niches and interspecific interactions. One commonly proposed mechanism to achieve functional complementarity is belowground vertical niche complementarity between shallow-rooting and deep-rooting species (Berendse, 1982;von Felten and Schmid, 2008).

When grown in monoculture, a species with roots that mainly occupy the shallow soil layers (shallow-rooting species) will be expected to mainly utilise water and nutrients from shallow soil layers (since they have no or very little roots in deep layers). Deep-rooting species in monoculture have roots that occupy deeper soil layers and can also access water and nutrients from deeper soil layers. However, the thick taproots of these species generally have a low root length density (cm root length per cm^3 soil volume) and, thus, tend to have lower resource uptake from shallow soil layers. A combination of these two types of species with their complementary strengths in mixture could result in a more complete exploitation of available soil resources than could be achieved by either species grown in monoculture. This complementarity could be enhanced if the belowground niche occupation of a given species would move away from zones of intense resource competition with neighbours when grown in mixture (von Felten et al., 2009;Mommer et al., 2010). In such a scenario, deep-rooting species can be expected to root even deeper and shallow-rooting species even shallower when grown in mixtures as a result of the shift in vertical soil niche occupation in mixtures compared to monocultures. Ultimately, this total increase in utilisation of water and nutrients may lead to higher above-ground biomass production than expected from a combination of monoculture performances.

However, the majority of research in grassland systems has focussed on aboveground responses, since the measurement of belowground plant biomass is much more labour intensive. Also, it is very hard to distinguish the roots of different species grown in mixtures in the field (see Mommer et al., 2008). Even when the roots of different species are identified, the presence and abundance of roots is not necessarily equivalent to root activity (Kulmatiski and Beard, 2013). Thus, insight into and evidence of belowground vertical niche complementarity and niche shifts of individual species in response to drought and interspecifc interactions is very limited.

Work is on-going to address these methodological challenges. Recently, ^{15}N tracers have been used to study nitrogen uptake patterns from different soil depths in grasslands with varying diversity levels (Pirhofer-Walzl et al., 2013;von Felten et al., 2009). As a measure of soil water utilisation, the natural abundance of $\delta^{18}O$ in soil and plant water can be used to measure the depth of water uptake of individual species (Durand et al., 2010;Dawson and Ehleringer, 1993;Nippert and Knapp, 2007a;Asbjornsen et al., 2008). Enabled by these methodological advances, new insights into these processes can yield important information with which to predict both the effect of future climate change on grassland production, and to better design agricultural systems with improved resource utilisation and resistance to drought.

The objective of this study was to use the natural abundance $\delta^{18}O$ isotope method to assess the effect of experimentally-induced drought on the depth of water uptake of shallow-rooting and deep-rooting species in intensively-managed grassland mixtures and monocultures. We tested the following hypotheses: 1) summer drought will result in a shift of water uptake to deeper soil layers, 2) deep-rooting species take up a higher proportion of water from deeper soil layers relative to shallow-rooting species, which is expected to give them an advantage under drought conditions, and 3) interspecific interactions result in a shift in the depth of water uptake of individual species grown in mixtures compared to monocultures. We hypothesise that water uptake by shallow-rooting species will become shallower when grown together with deep-rooting species and vice versa, resulting in reduced niche overlap.

1 Materials & Methods

1.1 Site and maintenance

We report measurements from two experiments that were conducted at Tänikon Research Station, Aadorf (47°48’N, 8°91’E) and Reckenholz, Zürich (47°43’N, 8°53’E) in Switzerland (Table 1). The experimental site at Tänikon was situated on a brown earth (topsoil sandy loam, subsoil clay) and the site at Reckenholz was a cambisol (topsoil 20-30% clay, subsoil 30-40% clay). Swards were sown in August 2010 and 2011 (Tänikon and Reckenholz, respectively) on 3 m × 5 m plots. Plots were cut seven times per year in Tänikon (2011), including a clearing cut in April, and six times in Reckenholz (2012). Plots received 145 and 200 kg N ha^{-1} yr^{-1} (Tänikon and Reckenholz, respectively) split over five applications, and enough P and K as to be non-limiting for intensively managed grassland.

1.2 Experimental design

Four grassland species were selected based on their expected rooting depth: two shallow-rooting species, *Lolium perenne* L (*L perenne*) cultivar (cv.) Aligator and *Trifolium repens* L (*T repens*) cv. Hebe, and two deep-rooting species, *Chicorium intybus* L (*C intybus*) cv. Puna II and *Trifolium pratense* L (*T pratense*) cv. Pastor in Tänikon and cv. Dafila in Reckenholz. *L perenne* and *T repens* generally have the bulk of their roots in the top 10 cm, whereas *C intybus* and *T pratense* have tap roots that allow access to deeper soil depths (Brown et al., 2003;Black et al., 2009). All four species were sown in four monocultures and in one mixture with equal proportions of all four species, resulting in five different plant communities. Monocultures of *L perenne*, *C intybus*, *T repens* and *T pratense* were sown with 30, 6.5, 16 and 16.4 kg germinable seeds ha^{-1}, respectively.

Using rainout shelters, half of the plots were subjected to a drought treatment of 10 and 9 weeks duration in Tänikon 2011 and Reckenholz 2012, respectively (spanning two regrowth periods, Table 1), with three replicate plots per treatment. The tunnel-shaped shelters consisted of steel frames of 5.5 m × 3 m × 1.4 m height, covered with 1.5 µm thick transparent plastic foil (Gewächshausfolien-Zuschnitt UV4, 190my) with the opposing ends left uncovered. Gutters were installed to prevent the water from flowing onto adjacent plots, and a ventilation opening at the top and bottom 50 cm was included to stimulate air circulation and minimise temperature increases underneath the shelters.

Air temperature and relative humidity (RH) were measured at 80 cm height with combined temperature and RH probes (Decagon EHT durable RH / temperature sensor) in four mixture plots at Tänikon and six mixture plots at Reckenholz, respectively (both control and drought plots) and logged (EM50, Decagon). Rainfall for the two experiments was recorded at the weather stations located at Tänikon Research Centre and ART, Reckenholz, Zürich which were within 2 km distance of the experimental sites. Photosynthetically active radiation (PAR) was measured above the canopy of all plots subjected to drought (underneath the shelter) and of control plots on five occasions during the drought period using a ceptometer (AccuPAR LP80, Decagon Devices, USA).

1.3 Sampling

At the end of the drought period, dry matter yield of the aboveground biomass was determined by cutting a 5 m × 1.5 m strip at 7 cm height from the centre of each plot using a Hege plot harvester. The species proportions were determined by cutting a 50 cm × 50 cm square from the centre of each plot using electric shears and sorting the herbage into the four sown species, dead material and unsown species. All herbage samples were oven-dried in a forced air oven at 60^{o}C for 48 hours to determine the dry matter content.

δ^{18}O Natural abundance of soil water and plant water was used to assess the depth of water uptake of individual species. The lower evaporation rate of heavy isotopes increases the concentration of ^{18}O in water at the soil surface. Diffusion of the isotopes in soil then follows from the top of the profile downwards. This results in a vertical gradient in isotopic composition of water in the soil (Durand et al., 2007). No isotopic fractionation occurs during soil water uptake by root systems, and therefore the composition of plant xylem water is an indicator of the mean depth of water uptake (Dawson et al., 2002).

Approximately one week before the end of the drought period, crown roots and stem bases (up to 1.5 cm above soil level) were collected from five to eight tillers (depending on tiller weight) of all four sown species. Samples were taken from two replicate plots of all treatment combinations. For *L perenne*, the outer sheath, which may be subject to transpiration and therefore have an altered δ^{18}O signal (Durand et al., 2007) was removed. At the same time, three 2 cm diameter soil cores were taken to 40 cm depth per plot and divided into four segments for Tänikon 2011 (0-10, 10-20, 20-30 and 30-40 cm) and five segments in 2012 (0-10 cm segment split into 0-5 cm and 5-10 cm). All samples were taken in the core plot area, excluding the outer 1 m border of the plot. All plant samples and a bulked sub-sample of the soil material were stored frozen in airtight glass vials (Exetainers, Labco, UK). The remainder of the soil material was oven-dried at 100^{o}C for 48 hours to determine the soil moisture content (SMC). Water from the soil and plant samples was extracted using cryogenic vacuum distillation (Ehleringer and Osmond, 1989).

Water samples were analysed for oxygen 18 isotopes at the Boston University Stable Isotope Laboratory on a MultiFlow (GV Instruments, Lyon, France) interfaced to an IsoPrime isotope ratio mass spectrometer (GV Instruments, Lyon, France). The procedure is based on the headspace equilibration technique (Socki et al., 1992). One hundred mL of water was placed in a Labco vial and capped. The vials were placed in a Gilson Autosampler and flushed with a mix of 5% CO_2/95% He, and then allowed to equilibrate for 8 hours at 40°C. During this time the oxygen isotopes in the water fully exchange with the oxygen in the CO_2. The headspace was subsampled and measured against a reference CO_2 gas. Samples were calibrated using IAEA standards and normalized to the V-SMOW/SLAP scale. Check standards were run every 10 samples to insure quality control and to correct for drift (if needed) and precision was usually 0.1 permil or better.

1.4 Data analysis

We used two methods to assess the ^{18}O data. Firstly, we used the direct inference approach (Asbjornsen et al., 2008;Brunel et al., 1995) to determine the mean soil depth from which each of the four species was extracting water. In this approach, the $\delta^{18}O$ isotopic signature of plant stem water is compared with the $\delta^{18}O$ signature of water at varying depths in the soil profile to determine at which depth the two values correspond to each other (Fig. B1). This soil depth is interpreted as the mean depth from which the plant extracted its water during the preceding time period. This approach assumes that roots preferentially take soil water from a single depth zone during any given period of time (Asbjornsen et al., 2007).

Secondly, we applied the IsoSource stable isotope mixing model (Phillips and Gregg, 2003) to quantitatively determine the proportional contribution of each of the sources (i.e. 4-5 soil depth intervals, as in Methods) to the plant stem water $\delta^{18}O$ signature. In this method, all possible combinations of each source contribution (1-100%) were examined in 1 % increments. Combinations of source contributions that summed to the observed plant stem $\delta^{18}O$ signature within a small tolerance (1 %) were considered to be feasible solutions. Based on this set of all feasible solutions, the frequency distribution, mean and 1-99th percentile range of the potential proportional contribution of each source were determined (for details, see Phillips and Gregg, 2003). Uncertainty associated with the proportional contributions from the 0-5 and 5-

10 cm layer (Reckenholz 2012) was high (i.e. the range of potential source contributions was relatively large). Therefore we used the a posteriori approach outlined by Phillips *et al.* (2005) to combine the 0-5 cm and 5-10 cm layer into one single layer (0-10 cm). To this end, we imported the output files from the IsoSource model containing all the feasible source contribution solutions into Excel, and created the aggregate 0-10 cm soil layer as the sum of the 0-5 and 5-10 cm soil layer. Subsequently, we calculated the new mean and range for the aggregated 0-10 cm layer. Both the direct inference and the IsoSource method assume that the only water source was soil water in the 0 to 40 cm soil depth interval.

For Tänikon 2011, all the plant samples of *L perenne*, *T repens* and *C intybus* and one sample of *T pratense* (control, mixture, replicate 1) were contaminated during the cryogenic vacuum distillation process due to a faulty vacuum, and therefore we only present the results for *T pratense* for Tänikon 2011. To avoid the risk of over-interpreting the data and to increase the comparability with the direct inference approach we focus on the proportional contribution to water uptake from the 0-10 cm soil depth interval (instead of all four soil depth intervals) calculated by the IsoSource model.

We aimed to assess whether the vertical soil niche occupation of different species became less similar in mixture compared to monoculture (hypothesis 3). To this end, we calculated the proportional similarity index (PS) (Colwell and Futuyma, 1971) of the proportional contribution to water uptake of the 0-10 and 10-40 cm soil depth interval (p_i), between pairs of species (species 1 and 2) for the Reckenholz 2012 data.

$$\mathrm{PS} = 1 - 0.5\sum_{i=1}^{n}\left|p_{1i} - p_{2i}\right| \tag{1}$$

The value of PS is minimum (0) when there is no overlap of the proportional water contribution of the two soil layers between two species within a species pair, and is maximum (1) when there is complete overlap.

1.5 Statistical analysis

Data were analysed by analysis of variance (ANOVA), considering the within-plot correlation of soil depth data and species where necessary by specifying a random plot grouping factor, and subsequent testing using a linear mixed model (Pinheiro and Bates, 2009). The full models consisted of all main effects and higher-order interactions, and were run separately for the two experiments. Differences among treatment levels were analysed based on model contrasts.

Main factors for soil moisture content and δ^{18}O values of the soil were water supply (drought and control), community (*L perenne* monoculture, *T repens* monoculture, *C intybus* monoculture, *T pratense* monoculture and the equi-proportional mixture) and soil depth interval (0-10, 10-20, 20-30 and 30-40 cm) (Table A1). With respect to dry matter yield of the aboveground biomass, main factors were water supply and community (Table A2), while for the species' proportions in the mixture, main factors were water supply and species (*L perenne, T repens, C intybus, T pratense*) (Table A3). The main factors for the mean inferred depth of water uptake (direct inference approach) and the proportional contribution to plant water uptake of the 0-10 cm soil depth interval (IsoSource model) were water supply, diversity (monoculture, mixture) and species (the latter for 2012 only) (Table A4). For the proportional similarity (Eq. 1) the main factors were water supply, diversity and species pair (*L perenne–T repens, L perenne–C intybus, L perenne–T pratense; T repens–C intybus, T repens–T pratense* and *C intybus–T pratense*) or rooting-depth pair (Shallow, Mixed, Deep) (Table A5), in which "Mixed" refers to species-pairs consisting of a shallow- and deep-rooting species. All statistical analyses were carried out using the statistical software R (R Development Core Team, 2012).

2 Results

2.1 Growth conditions

During the drought period, a total of 306 and 247 mm of rain was excluded from the drought plots, which corresponded to 33% and 21% of the total annual rainfall for Tänikon 2011 and Reckenholz 2012, respectively (Table 1). The mean air temperature under the shelters was slightly higher (0.8 ^{o}C and 0.4 ^{o}C increase for Tänikon 2011 and Reckenholz 2012, respectively), whereas the relative humidity was slightly lower (-0.02 % and -0.01 %, respectively) (Table 1). The incoming PAR was 11 and 28 % lower underneath the shelters compared to control plots in Tänikon 2011 and Reckenholz 2012, respectively (Table 1). Under control conditions, the soil moisture content ranged from 0.33 and 0.20 g water per g dry soil in the 0-10 cm soil depth interval to 0.28 and 0.18 g water per g dry soil in the 30-40 cm soil depth interval for Tänikon 2011 and Reckenholz 2012, respectively. In both experiments, soil moisture content was significantly lower under drought compared to control conditions ($p < 0.001$). The difference in soil moisture content between control and drought was on average 0.15 and 0.11 g water per g dry soil in the 0-10 cm soil depth interval, but was only 0.03 and 0.06 g water per g dry soil in the 30-40 cm soil depth interval for Tänikon 2011 and Reckenholz 2012, respectively, resulting in a significant water supply × depth interaction ($p < 0.001$, Fig. 1a, b and Table A1).

2.2 Aboveground biomass

There was a significant reduction in herbage dry matter yield under drought conditions of 18 % ($p < 0.05$) for Tänikon and 41% for Reckenholz ($p < 0.001$) (Table 2 and A2). Overall, there was a significant effect of community ($p < 0.001$) on the herbage dry matter yield. Yield values were highest for the *T pratense* monoculture followed by the equi-proportional mixture and lowest for the *L perenne* monocultures (mean dry matter yield of 2854, 2211 and 671 kg ha^{-1}, respectively). The yield reduction (for Tänikon 2011 and Reckenholz 2012, respectively) under drought conditions was highest for *L perenne* (65 % and 76 %) and *C intybus* (37 % and 62%), and lowest for *T pratense* (2 % and 21 %) and in 2012 this resulted in a borderline significant ($p = 0.08$) community × water supply interaction (Table 2 and A2).

In mixture, *T pratense* was always the dominant species with an average proportional contribution to dry matter yield of 0.55, whereas the proportions of the other species were on average 0.20, 0.08 and 0.08 for *L perenne, T repens and C intybus*, respectively, resulting in a significant ($p < 0.001$) effect of species on the species proportion (Table 2 and A3).

2.3 Soil water $\delta^{18}O$

Soil water δ^{18}O was significantly more negative under drought compared to control conditions ($p < 0.001$, Table A1). Generally, the δ^{18}O values of soil water were highest (least negative) in the top 10 cm and values significantly declined at deeper soil depth intervals ($p < 0.001$, Fig. 1c, d and Table A1). The difference between control and drought plots was smaller at shallow compared to deep soil depth intervals, resulting in a significant water supply × depth interaction ($p < 0.001$, Fig. 1c, d and Table A1).

2.4 Depth of water uptake

2.4.1 *Comparison of methods*

The mean inferred soil depth of water uptake was 13 cm (Fig. 2a-e) and varied from 5 cm for *T repens* (control, mixture, see Fig. 2c) to 26 cm for *C intybus* (drought, monoculture, Fig 2d). The mean proportional contribution of the 0-10 cm soil layer to plant water uptake ($PCWU_{0\text{-}10}$) was on average 0.48 (Fig. 2f-j) and ranged from 0.07 to 0.88, corresponding to the treatments with the lowest and highest depth of soil water uptake, respectively. In general there was a good correlation between the two methods to assess the depth of water uptake based on $\delta^{18}O$ analysis (r = 0.86, Fig. 3), but the IsoSource method resulted in more statistically significant effects, as outlined below and in Table A4. There was a strong agreement between the *T pratense* data from Tänikon 2011 and Reckenholz 2012 (Fig. 2), giving confidence in the robustness of the dataset.

The proportional contribution to plant water uptake of the 10-20 cm, 20-30 cm and 30-40 cm soil depth interval was very similar and on average 0.16, 0.17 and 0.19, respectively (Fig. C1). Therefore, we present a two-pool model, in which the 10-40 cm soil depth interval is the mirror image of the 0-10 cm soil depth interval.

2.4.2 *Drought effect*

When grown in monoculture, the $PCWU_{0\text{-}10}$ of *L perenne*, *T repens* and *C intybus* was reduced by 0.35 ($p < 0.05$), 0.29 ($p = 0.06$) and 0.17 ($p = 0.27$), respectively under drought compared to control conditions, indicating a shift to deeper soil layers (Fig. 2f-j and 4a). In contrast, the $PCWU_{0\text{-}10}$ of *T pratense* grown in monoculture increased by 0.27 ($p < 0.05$) and 0.20 ($p = 0.17$) in 2011 and 2012, respectively.

When grown in mixture, the effect of drought on the $PCWU_{0\text{-}10}$ of *L perenne* and *T repens* was in the same direction but less pronounced compared to when grown in monoculture (reduction in $PCWU_{0\text{-}10}$ of 0.27 ($p = 0.08$) and 0.17 ($p = 0.27$), respectively (Fig. 4b). However, the drought effect on the $PCWU_{0\text{-}10}$ of *T pratense* was inversed in mixture compared to monoculture as $PCWU_{0\text{-}10}$ decreased with 0.23 ($p < 0.05$) and 0.28 ($p = 0.07$), for 2011 and 2012 respectively, resulting in a borderline significant ($p = 0.05$) water supply × species × diversity interaction (Fig 2f-j, 4a, b and Table A4).

2.4.3 Differences among species' monocultures

There was a significant effect of plant species in monoculture ($p < 0.01$, Table A4, Fig. 2) on the inferred depth of water uptake and the $PCWU_{0\text{-}10}$, when grown in monoculture. For the two shallow-rooting species *L perenne* and *T repens*, water uptake was concentrated in the top 0-10 soil depth interval (10 cm depth and $PCWU_{0\text{-}10} = 0.53$ averaged for both species and water supply treatments). In contrast, the deep-rooting *C intybus* mainly relied on deeper soil layers (25 cm depth and $PCWU_{0\text{-}10} = 0.16$). Surprisingly, water uptake of the deep-rooting species *T pratense* was comparable or even more shallow than for the shallow-rooting species in both experiments (on average 9.6 cm depth and $PCWU_{0\text{-}10} = 0.68$, Fig. 2).

2.4.4 Effect of interspecific interactions in mixtures

T pratense showed a strong shift to deeper soil water uptake in response to being grown in mixture compared to monoculture. The $PCWU_{0\text{-}10}$ averaged over the two experiments decreased by 0.17 and 0.67 under control and drought conditions, respectively (Fig 2 and 4c, d). Similarly the inferred depth of water uptake increased from 12.8 to 16.8 cm and from 6.4 to 17.0 cm under control and drought conditions, respectively. The opposite was found for the other species, which tended to move their water uptake to shallower soil depth intervals resulting in a significant ($p < 0.05$ and $p < 0.01$ for depth of water uptake and $PCWU_{0\text{-}10}$, respectively) species × diversity interaction (Table A4 and Fig. 2 and 4c, d).

We calculated the proportional similarity (Eq. 1) of the proportional water contribution of the different soil depth intervals to assess whether this shift in response to diversity resulted in reduced similarity between shallow- and deep-rooting species in mixtures compared to monocultures. The proportional similarity of mixed- and shallow-rooting species pairs was the same in monoculture compared to mixture, whereas the proportional similarity of the deep-rooting species pair *C intybus* – *T pratense* actually increased in mixtures compared to monocultures (significant rooting depth pair × diversity interaction, $p < 0.001$, Fig. 5, Table A5).

3 Discussion

3.1 The effect of drought on the depth of water uptake

In line with our hypothesis, under drought compared to control conditions, *L perenne*, *T repens* and *C intybus* substantially decreased the proportional water uptake from the 0-10 cm soil depth interval when grown in monoculture, and instead increased the proportional uptake from deeper and less dry soil layers. In contrast, *T pratense* grown in monoculture actually shifted its proportional water uptake to more shallow soil depths under drought compared to control conditions which was consistent for the two experiments. We do not have a clear explanation for this upward shift and it is contrary to general expectation, but the effect was clear and consistent for both experimental sites/years. The soil moisture content and distribution throughout the soil profile was similar to the other plant communities (no significant effect of community, Table A1), and does not indicate increased water extraction from the top soil layer. Of all the species, the dry matter yield of *T pratense* was least affected by the drought treatment (Table 2). Changes in pre-dawn leaf water potential in response to drought were similar for *T pratense* and *T repens* and provided no evidence for differences in stomatal control (unpublished data).

There are very few data on the effect of drought on the depth of water uptake in grassland systems in the literature, and the findings are highly variable. Grieu et al. (2001) reported a substantial increase in the soil water uptake from deeper soil layers by *L perenne* and *T repens* seedlings grown in containers under moderate soil water deficit. In contrast, using the natural abundance δ^{18}O technique, Prechsl (2013) found that mixed C3 grassland communities (dominated by *Phleum pratense*, *Lolium multiflorum*, *Poa pratensis*, *Taraxacum officinale*, *Trifolium repens* and *Rumex obtusifolius*) subjected to artificial summer drought relied strongly on the topsoil (0-10 cm) for water (about 56%) during drought, whereas the roots of plants that were not subjected to drought shifted to deeper soil layers during the summer months and relied less on the topsoil (about 30%). These results were reflected in changes in root biomass at the different soil depth intervals. Similarly, a number of studies using natural abundance δ^{18}O techniques (Asbjornsen et al., 2008;Nippert and Knapp, 2007a;Nippert and Knapp, 2007b) or deuterium oxide labelling techniques (Kulmatiski and Beard, 2013) focussing on C4 grasses and herbaceous species grown in combination with shrubs or trees, show that the grasses tended to solely rely on shallow soil water, whereas shrubs and trees are more dependent on deeper soil water (30 cm to > 150cm soil depth) under natural seasonal drought conditions.

Also, reported responses of root growth to drought are variable. For example, Garwood and Sinclair (1979) reported a slight increase in percentage of *L perenne* root length in the 0-10 cm soil depth under non-irrigated compared to irrigated plots (72 and 63 % respectively) whereas Skinner (2008) found that mixtures of *L perenne* and *T repens* and *L perenne*, *T repens* and *C intybus* had decreased root counts in the upper 40 cm and increased root counts at lower depths in drought stressed plots compared to control plots.

There are a number of reasons that may explain the different findings. Firstly, the severity and length of the drought will affect the plant water availability throughout the soil profile and therefore the impact of the drought. For example, during the drought period in our study, water availability was more limited in the 0-10 cm soil layer compared to the deeper soil layers (Fig 1a, b). During the natural seasonal drought reported by Kulmatiski and Beard (2013), the water availability under drought conditions did not increase with soil depth within the rooting zone, and therefore, under those conditions, there is no obvious benefit for relatively shallow rooting species to invest in root growth to explore deeper layers. Other studies do not include sufficiently detailed information on soil water availability throughout the profile (Nippert and Knapp, 2007a;Asbjornsen et al., 2008), which emphasises the importance of measuring and reporting the soil moisture content throughout the rooting zone (Vicca et al., 2012).

Secondly, differences in species and plant communities may affect the response to drought. Our results show that plants grown in mixtures can have a different response to drought compared to their response in monoculture. For example, in contrast to its unexpected increase in the $PCWU_{0-10}$ under drought conditions in monoculture, *T pratense* decreased $PCWU_{0-10}$ in response to drought when grown in mixture (Fig 4b). Also, for *T repens* and *C intybus* the response to drought was much less pronounced when grown in mixtures compared to monocultures. Most of the studies above (Nippert and Knapp, 2007a;Nippert and Knapp, 2007b;Asbjornsen et al., 2008;Kulmatiski and Beard, 2013;Prechsl, 2013) only look at the response in mixtures and not monocultures. Also they are based on very different systems of relatively shallow-rooting grasses and herbaceous species with deep-rooting shrubs or trees (with a much deeper rooting depth) (Nippert and Knapp, 2007a;Nippert and

Knapp, 2007b;Asbjornsen et al., 2008;Kulmatiski and Beard, 2013). In such systems, there is a competitive benefit for grasses to focus water uptake in surface soils due to their fibrous root morphology and a greater ability to respond to pulses in water availability (Nippert and Knapp, 2007b;Caldwell and Richards, 1986).

3.2 Depth of water uptake of shallow- and deep-rooting species

The two shallow-rooting species in this study (*L perenne* and *T repens*), had a significantly higher proportional water uptake from the shallow (0-10 cm) soil depth interval compared to the deep rooting species *C intybus*, showing that these deep- and shallow-rooting species occupy distinctly different vertical niches in relation to water uptake. This is in line with work by Pirhofer-Walzl et al. (2013) who reported that the proportion of ^{15}N uptake from shallow root layers (0-40 cm) decreased in the order *L perenne* > *T repens* > *C intybus*.

However, in contrast to our expectation, the depth of water uptake of the deep-rooting species *T pratense* grown in monoculture tended to be shallower than the two shallow-rooting species. We classified *T pratense* as a deep-rooting species because it has tap roots, which can access deeper soil layers (Black et al., 2009). However, the d^{18}O results indicate that the presence of roots in a particular soil layer is not necessarily equivalent to root activity, which has been confirmed in other studies (Nippert and Knapp, 2007a;Kulmatiski and Beard, 2013). In the current study, the deep-rooting *T pratense* actually had shallow water uptake in monoculture, and it would seem that the plant rooting depth determines the potential range or plasticity in depth of water uptake rather than the actual depth of water uptake. Indeed, the plasticity of *T pratense* in relation to depth of water uptake was very large and the $PCWU_{0\text{-}10}$ ranged from 0.13 to 0.82, which could have clear competitive advantages. Under control conditions, when water availability was not limited, uptake from shallower soil layers (compared to deeper) would be beneficial to the plant since there would be a shorter path length for transport, and therefore a reduced gradient in water potential required for movement. The fact that the other deep-rooting species *C intybus* relied on deeper soil water under control conditions even when grown in monoculture, indicates that *C intybus* roots may have a lower plasticity for water uptake from different soil depths compared to *T pratense*.

This research shows that classification of species according to rooting depth may be of limited value, as the depth of main root activity depends on the specific conditions. Similarly, Durand et al. (1997) demonstrated that *L perenne* could extract water from very similar depth as *F arundinacea*, a renowned deep rooted species.

3.3 Shift in depth of water uptake in mixed communities

We hypothesised that shallow-rooting species would move their water uptake to more shallow soil depth intervals and deep-rooting species to deeper soil depth intervals in mixtures compared to monocultures. Our results partly support this hypothesis as both shallow-rooting species *L perenne* and *T repens* tended to take up water from shallower soil depth intervals when grown in mixtures compared to monocultures (Fig. 4c, d). Additionally, the deep-rooting species *T pratense* dramatically increased its proportional water uptake from deeper soil layers in mixtures compared to monocultures, particularly under drought conditions. However, the opposite was true for the other deep-rooting species *C intybus*. The diverging response of *C intybus* may be explained by the fact that *T pratense* was the dominant deep-rooting species (aboveground species' proportions were on average 55 and 8 % for *T pratense* and *C intybus*, respectively), which may have outcompeted *C intybus* at the deeper soil levels forcing it upward instead.

This niche differentiation did not result in a reduction of niche overlap between deep- and shallow-rooting species, as the proportional similarity of PCWU of the different soil depth intervals between shallow- and deep-rooting species did not decrease in mixtures compared to monocultures (Fig. 5). This was the result of 1) the increase in proportional similarity between *C intybus* and the shallow-rooting species and 2) the net effect of the strong change to deeper depth for *T pratense* was limited under drought conditions, since it started from a very shallow depth when grown in monoculture (Fig. 4c, d). However, the strong dominance of *T pratense* compared to the other species in terms of aboveground biomass, may limit the value of the proportional similarity as an indication of community resource utilisation, since the contributions of all species are equally weighted. A substantial decrease in the proportional similarity of a pair of sub-dominant species may not affect the community resource utilisation (von Felten et al., 2009).

Our results show very little evidence for shifts in the vertical niche of shallow and deep rooting grassland species in mixtures compared to monocultures, and we could find none relating to depth of water uptake. Berendse (1982) showed that *Plantago lanceolata* acquired nutrients from deeper soil layers when grown together with the shallow-rooting grass *Anthoxanthum odoratum* than when grown in monoculture. Using ^{15}N tracers to examine N partitioning, von Felten et al. (2009;2012) showed that both niche breadth of individual species and niche overlap among species decreased with increasing species richness. Using a DNA-based technique to compare species-specific root distribution, Mommer et al. (2010) found that even though root biomass was significantly larger in a four-species grassland mixture compared to monocultures, this was not due to a shift in vertical niche distribution. In contrast, the rooting depth of the community tended to decrease, rather than increase in mixtures compared to monocultures.

3.4 Higher drought resistance for deep-rooting species?

There was no clear link between interspecific differences in the depth of water uptake and the drought resistance (reduction in aboveground biomass under drought compared to control conditions). In line with our hypothesis, the shallow-rooting *L perenne* was most affected by drought, however, *C intybus*, the species with the deepest water uptake also had a very large reduction in the aboveground biomass under drought conditions. Interestingly, *T pratense*, which was least affected by drought, also had the greatest plasticity in depth of water uptake. This suggests that there may be an indirect effect of rooting depth on drought resistance, as it determines the potential plasticity in the depth of water uptake (as discussed in section 3.2). Additionally, other mechanisms such as species drought tolerance (Chaves et al., 2003) and nutrient availability (i.e. restrictions in available nitrogen under drought conditions, Hofer et al., 2013) are likely to affect the impact of drought on herbage dry matter yield.

3.5 Does belowground vertical niche complementarity in depth of water uptake underpin the diversity effect in aboveground biomass?

The δ^{18}O results shows that at least some of the species in this experiment occupied a distinctly different vertical niche in relation to proportional water uptake from different soil depth intervals and also showed shifts in niche occupation in response to diversity. However, the natural abundance $\delta^{18}O$ method does not provide a quantitative measure of water uptake, as it is limited to measuring the proportional uptake from the different soil depth intervals. Therefore, it remains unknown whether total water uptake of the mixture was increased or not. Recent studies using ^{15}N tracers allowed the measurement of total nutrient uptake in addition to the proportional contribution (Pirhofer-Walzl et al., 2013;von Felten et al., 2009;Kahmen et al., 2006;von Felten et al., 2012). These studies reported that even though plants did occupy complementary spatial niches, this did not result in increased community nitrogen uptake in more diverse communities. von Felten et al. (2009) reported that the decrease in niche breadth and niche overlap mostly occurred among subordinate species or pairs of subordinate and dominant species, rather than among dominant species. Therefore, they concluded that niche differentiation with respect to N uptake from different chemical forms and soil depths was not a major driver of positive diversity–ecosystem functioning relationships in their experiment, but facilitated the co-existence of sub-ordinate species. In contrast, in the current study, the main shift in depth of water uptake in mixture compared to monoculture was for the dominant species, *T pratense*.

In the current study, the biomass results showed that the observed mixture yield was higher than the predicted mixture yield (over-yielding), indicating a diversity effect. Our results suggest that differences in the depth of water uptake between species may have resulted in vertical niche complementarity in the depth of water uptake between deep-and shallow-rooting species, which may have contributed to this over-yielding. However, due to the lack of data on quantitative water uptake from different soil depths, we cannot provide direct evidence for this. Additionally, other factors, such as vertical soil niche complementarity for nutrients, or interactions between legumes and non-legumes, soil-biotic factors or a combination of factors may have also contributed to the diversity effect.

3.6 Methodology

The use of natural abundance $\delta^{18}O$ to assess the effect of drought on patterns of water use by co-occurring species worked well. We observed a clear soil gradient, which is common in soil depths up to 50 cm for perennial grass systems (Asbjornsen et al., 2007;Nippert and Knapp, 2007a). The δ^{18}O signal in soil water was more negative for drought compared to the control treatment. This might be the result of reduced evaporation from the drought plots, related to the reduction in incoming radiation under the drought shelters (reduction in incoming PAR of 10-28%). However, this effect is somewhat reduced because vegetation ground cover tended to be lower and temperature higher under drought conditions. Monthly rainfall d^{18}O isotopic composition data from the Swiss National Network for the Observation of Isotopes in the Water Cycle (ISOT) (Schürch et al., 2003) show that the d^{18}O of rainwater was less negative during the drought period compared to the preceding months (difference of 2.1 and 2.9 during 2011 and 2012, respectively, Fig. D1). Therefore, the control plots may have become relatively enriched due to the increased enrichment of rain water during the drought period.

In general, there was a good correlation between the depth of water uptake estimated through the direct inference method and the IsoSource model ($r = 0.86$), and the trends in relation to species, diversity and water supply were similar for both methods. However, the treatment effects were more pronounced for the IsoSource model, and therefore resulted in more significant effects. It should be noted that these treatment effects are based on the mean of the frequency distribution, and that the full range (1-99 percentile) of the possible proportional contribution showed overlap for some treatments (Fig. 2f-j). The two main drawbacks of the direct inference approach are 1) the assumption that plants rely on water uptake of one single mean depth and 2) that the determination of this soil depth can be arbitrary, particularly in situations when there is no strong gradient in the soil δ^{18}O profile (Asbjornsen et al., 2007). An advantage of the IsoSource model over the direct inference method is the possibility to quantitatively describe relative contributions of water from the different soil depth intervals in a systematic way (Phillips and Gregg, 2003).

For practical reasons, the δ^{18}O sampling depth was limited to 40 cm soil depth. However, it is not unlikely that water uptake from below this depth occurred (Skinner, 2008;Pirhofer-Walzl et al., 2013;Garwood and Sinclair, 1979). This would not have affected the mean inferred depth of water uptake, as these values were all well above 40 cm (Fig 2a-e). In order to get an idea of the potential effect of limiting the soil sampling depth to 40 cm on the output of the IsoSource model, we re-ran the model with estimated δ^{18}O values for the 40-50 cm soil depth interval. We assumed that the decline in δ^{18}O with increasing soil depth would start to “level out” at this depth (see also Fig. 2), and estimated the δ^{18}O value for the 40-50 cm soil depth interval as the δ^{18}O value for 30-40 cm + (30-40 cm – 20-30 cm) / 2. The resulting estimates for PCWU$_{0-10}$ were highly correlated to the original estimates (r^2 = 0.99) and were marginally higher (0.51 instead of 0.49), as there was now more support for the relative reliance on shallow soil depths. As a result, adding an extra (estimated) depth to the IsoSource model input had no effect on the observed trends in response to drought and diversity.

In this experiment, we simulated summer drought by using rainout shelters for a period of 10 weeks, resulting in a significant reduction in soil moisture content in the drought plots. The use of shelters resulted in an increase in the mean air temperature (3%), soil temperature (8%) and a decrease in air relative humidity (-2%), which can potentially exacerbate the effect of rain-exclusion alone. De Boeck and Verbeeck (2011) showed that naturally-occurring drought was accompanied by similar changes in temperature (11.1%) and relative humidity (-11.6%). In contrast, the shelters reduced the incoming PAR (10-28%), which may have limited the water loss through evapo-transpiration in the drought treatment. This re-emphasises the importance of measuring and reporting the soil moisture content throughout the rooting zone (Vicca et al., 2012) as the main indicator of the severity of the drought treatment. In our experiment, soil moisture content was strongly reduced in the drought compared to the control plots, particularly in the top soil.

4 Conclusions

- The δ^{18}O natural abundance method provided new insights into the depth of water uptake of grassland species. There were large treatment effects on the depth of water uptake of *T pratense* in particular.
- In line with our hypothesis, monocultures of *L perenne*, *T repens* and *C intybus* moved their water uptake to deeper soil depth intervals in response to drought, However, *T pratense* in monoculture did the opposite, and we have no clear explanation for this.
- As expected, the two shallow-rooting species had higher proportional water uptake from the shallow soil depth interval compared to the deep-rooting *C intybus*. However, the deep-rooting *T pratense* grown in monoculture relied more on shallow soil water than the shallow-rooting species.

- As hypothesised, interspecific interactions in mixtures resulted in a shift in the depth of water uptake, which tended to become shallower for the shallow-rooting species and deeper for the deep-rooting *T pratense*. However, this did not result in a net reduction in proportional similarity (niche overlap) between shallow and deep-rooting species in mixture.
- There was no clear link between depth of water uptake and resistance to drought, as the species with the deepest water uptake (*C intybus*) was also most affected by drought. *T pratense*, which was least affected by drought, also had the greatest plasticity in depth of water uptake. This suggests that there may be an indirect effect of rooting depth on drought resistance, as it determines the potential plasticity in the depth of water uptake.

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Tables

Table 1. Overview of dates and micrometeorological conditions under drought and control treatment during the final week of the drought period in Tänikon 2011 and Reckenholz 2012.

	Tänikon 2011			Reckenholz 2012		
Sowing date	August 2010			August 2011		
Start drought period	16/06/2011			06/07/2012		
Duration drought period (weeks)	10			9		
Rain excluded during drought (mm)	306			247		
Rain exclusion (% of annual rainfall)	33%			21%		
	Control	Drought	% change	Control	Drought	% change
Relative humidity	0.79	0.77	-3	0.87	0.86	-1
Mean air temperature (^{o}C)	20.3	21.1	4	15.6	16.0	3
Maximum air temperature (^{o}C)	27.7	29.4	6	20.5	21.7	6
Mean soil temp. at 5 cm depth (^{o}C)	19.1	19.7	3	16.3	18.6	14
Photosynthetically active radiation (µmol m^{-2} s^{-1})	1115	998	-10	1611	1164	-28

Table 2. Average aboveground dry matter yield (kg ha^{-1}, SE in parenthesis, *n* = 3) of the plant communities and the proportion of dry matter yield of the sown species in the mixture under control and drought conditions during the final cut of the drought period in Tänikon 2011 and Reckenholz 2012 (See Table A2 and A3 for statistical significance of treatment effects).

	Dry matter yield (kg ha^{-1})					
	Tänikon, 2011			Reckenholz, 2012		
Community	Control	Drought	% change*	Control	Drought	% change
Monocultures						
L perenne	1355 (98.4)	479 (186.7)	-65%	682 (78.2)	166 (46.7)	-76%
T repens	1763 (34.5)	1523 (74.4)	-14%	1197 (128.9)	789 (80.0)	-34%
C intybus	1477 (179.8)	935 (78.3)	-37%	2062 (143.3)	787 (40.2)	-62%
T pratense	2841 (103.2)	2791 (149.5)	-2%	3232 (193.2)	2551 (358.8)	-21%
Predicted mixture**	2076 (109.1)	1462 (98.0)	-30%	1999 (183.3)	1396 (270.0)	-30%
Mixture	2113 (363.8)	2110 (378.4)	0%	2955 (187.4)	1665 (262.0)	-44%

	Proportion of dry matter yield of sown species in the mixture			
	Tänikon, 2011		Reckenholz, 2012	
Species***	Control	Drought	Control	Drought
L perenne	0.26 (0.04)	0.24 (0.01)	0.19 (0.09)	0.10 (0.03)
T repens	0.07 (0.02)	0.21 (0.06)	0.04 (0.02)	0.02 (0.01)
C intybus	0.13 (0.05)	0.06 (0.03)	0.06 (0.02)	0.08 (0.01)
T pratense	0.52 (0.03)	0.43 (0.04)	0.59 (0.23)	0.67 (0.16)
Dead	0.02 (0.01)	0.06 (0.02)	0.12 (0.10)	0.13 (0.12)

* (Drought - Control) / Control

**The predicted mixture yield is calculated based on the monoculture yields multiplied by the species' relative abundances (each species' proportional contribution to aboveground biomass) in the previous harvest.

***The proportion of unsown species in community dry matter yield was less than 0.0025

Figures

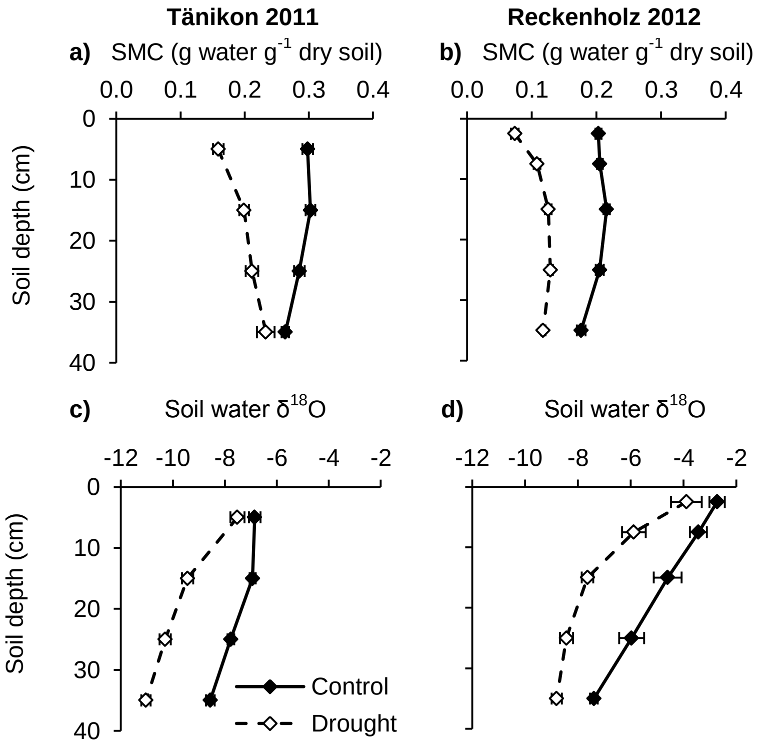

Figure 1. Mean (± SE) soil moisture content (SMC, g water g dry soil^{-1}) (a, b) and δ^{18}O value of the soil water extracts (c, d) throughout the soil profile for control and drought plots averaged across the different communities in Tänikon 2011 and Reckenholz 2012 (*n* = 10).

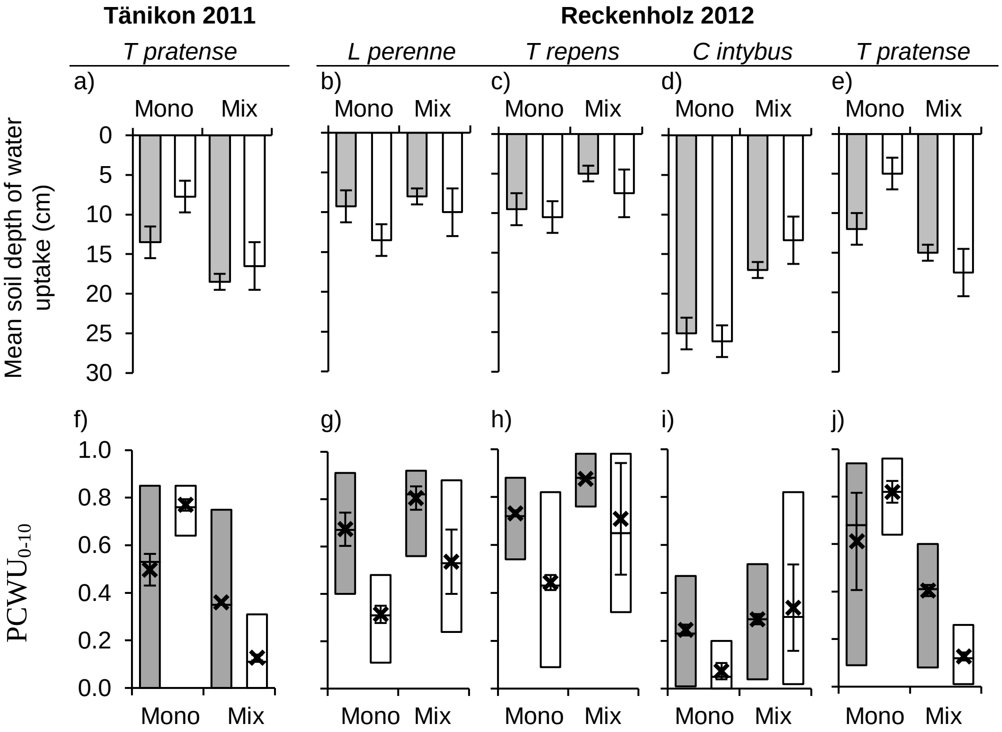

Figure 2. The mean soil depth of water uptake (cm, a-e) and the proportional contribution to plant water uptake of the 0-10 cm soil depth interval ($PCWU_{0-10}$) (f-j) of the two shallow-rooting (*L perenne* and *T repens)* and deep-rooting (*C intybus* and *T pratense)* species grown in monoculture (Mono) or mixture (Mix) under control (shaded bars) or drought (white bars) conditions in Tänikon, 2011 (*T pratense* only) and Reckenholz, 2012. The mean soil depth of water uptake is based on the direct inference approach (see Fig. S1). The proportional contribution to plant water uptake (f-j) is based on the frequency distribution output from the IsoSource model and lower, middle and upper boundaries of the bars represent the 1st percentile, 50th percentile and 99th percentile of the proportional contribution, respectively (See Fig. S2 for all soil depth intervals). The mean (×) and SE of the mean proportional contribution are also included (*n* = 2 in all cases except for Tänikon-*T pratense*-control-mixture and Reckenholz-*T repens*-control-mixture, where *n* = 1).

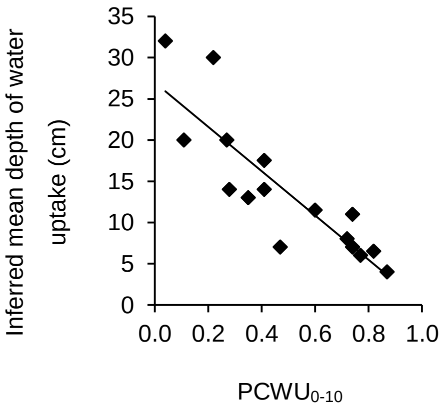

Figure 3. Correlation between the proportional contribution to water uptake of the 0-10 cm soil depth interval ($PCWU_{0\text{-}10}$) with the inferred mean depth of water uptake ($r = 0.86$)

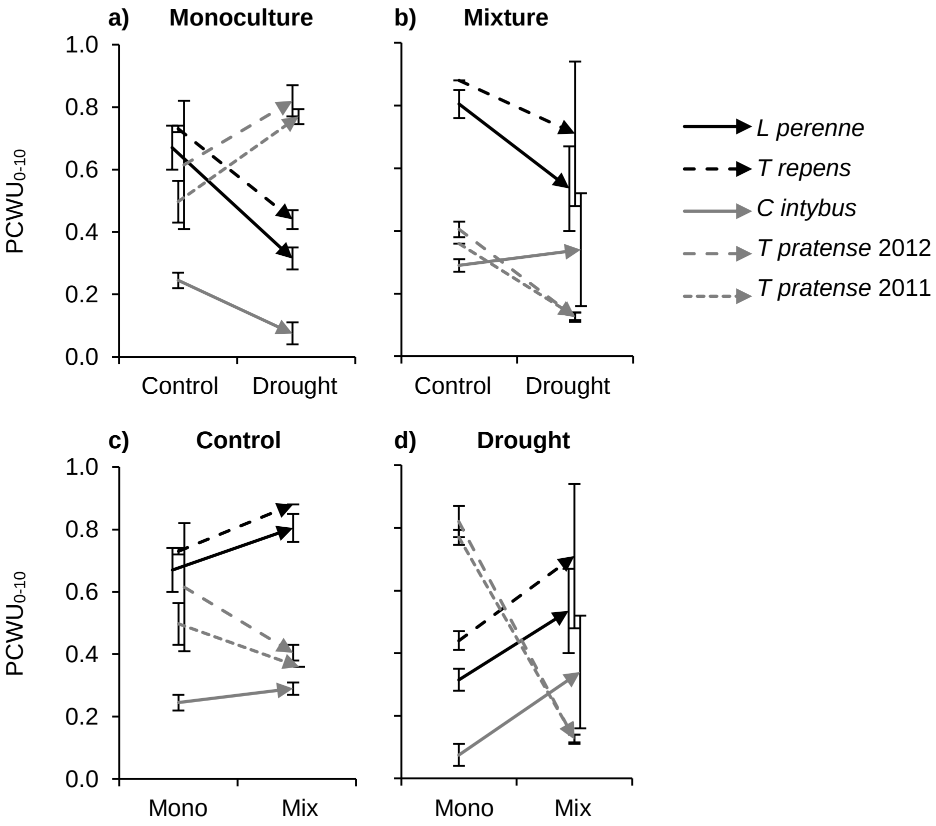

Figure 4. The shift in proportional contribution to plant water uptake from the 0-10 cm soil depth interval (PCWU$_{0\text{-}10}$) of shallow-rooting species (*L perenne* and *T repens*) and deep-rooting species (*C intybus* and *T pratense* 2011, 2012) as a result of water supply (a, b: control and drought) and diversity (c, d, monoculture and mixture). These graphs are based on the mean values presented in Fig. 2f-j, error bars represent one SE, *n* = 2 (for exceptions see Fig. 2).

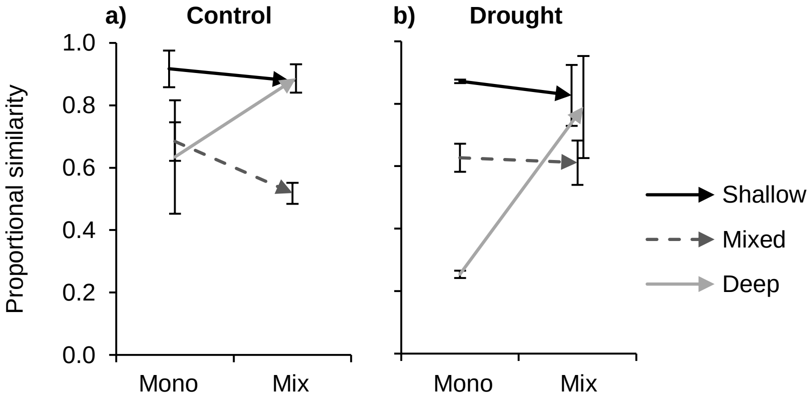

Figure 5. The proportional similarity (Eq. 1) of the proportional water uptake from the different soil depth intervals of shallow-rooting (*L perenne–T repens*), mixed-rooting (refers to combinations of shallow- and deep rooting species, and is the mean of *L perenne–C intybus*, *L perenne–T pratense; T repens–C intybus; T repens–T pratense*) and deep-rooting (*C intybus–T pratense*) species pairs grown in monoculture (Mono) or mixture (Mix) under a) control and b) drought conditions in Reckenholz 2012.