Crops grown in mixtures show niche partitioning in spatial water uptake

Journal Article

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RESEARCH ARTICLE

Crops grown in mixtures show niche partitioning in spatial water uptake

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Abstract

1. More diverse plant communities are generally more productive than monocultures. This benefit of species diversity is supposed to stem from resource partitioning of species in mixtures where different species use the resources spatially, temporally, or chemically in distinct ways. With respect to water, the simultaneous cultivation of crops with distinct water uptake patterns might reduce niche overlaps and thus result in higher productivity. However, little is known about whether and how spatial water uptake patterns of crop species differ among different planting arrangements and whether these changes result in increased niche partitioning and explain overyielding in mixtures.

2. Stable isotopes of water and a Bayesian model were used to investigate the spatial water uptake patterns of six different crop species and how these patterns change depending on the planting arrangement (monocultures vs mixtures). Niche overlaps and niche widths in spatial water uptake were compared among the different crop diversity levels and linked to productivity. Furthermore, spatial water uptake was related to competition intensity and overyielding in mixtures.

3. We found evidence for increased niche partitioning in spatial water uptake, and therefore complementary spatial root distributions of crop species, and higher expected productivity in mixtures compared to expected productivity in monocultures both due to inherent species-level differences in water uptake and plasticity in the water uptake pattern of species. We also found a significant relationship of competition and overyielding with observed patterns in spatial water uptake. These results suggest that competition was most intense in shallow soil layers and enhanced overyielding was related to a gradual increase of water uptake in deeper soil layers. Thus, overyielding might be related to a more complete spatial exploitation of available water sources.

4. *Synthesis.* Differences in spatial water uptake and niche partitioning of intercropped species, driven most likely by a complementary spatial root distribution, might explain why mixtures outperform monocultures. These findings underpin the potential of intercropping systems for a more sustainable agriculture with a more efficient use of soil resources and hence reduced input demands.

1 | INTRODUCTION

Resource partitioning of plants occurs both above- and below-ground (Barry et al., 2019) when plants use resources differently to avoid niche overlaps (Hutchinson, 1978). A crucial factor determining below-ground resource partitioning is the root distribution pattern. Under the condition that water is not limited along the whole soil profile, plant water uptake patterns are tightly coupled to root distribution patterns (Gardner, 1964) and can thus also be seen as a proxy to investigate spatial root distribution. At the same time, the rooting system of plants is highly plastic and roots segregate to avoid below-ground competition with other plants (Cabal et al., 2020; Schenk, 2006; Schenk et al., 1999). Indeed, plant species can shift to spatially different water sources upon competition with other species (Bartelheimer et al., 2010). Independent of whether resource partitioning is due to species inherently taking up resources in spatially, temporally, or chemically distinct ways or whether they have the ability to shift to an alternative source of water or other resources upon competition, communities with an increased level of resource partitioning tend to be more productive (Barry et al., 2019; Turnbull et al., 2016).

Concerning agricultural systems, simultaneously cultivating various crop species in the same field (i.e. intercropping) has many benefits such as reduced competition and complementarity effects which can lead to overyielding (i.e. higher yield in mixtures compared to monocultures) (Brooker et al., 2015; Li et al., 2014; Vandermeer, 1989, 1990). An underlying mechanism of overyielding in intercropping might be the distinct rooting patterns of crop species (Fan et al., 2016; Kutschera et al., 2009) and phenotypic plasticity leading to complementary rooting patterns, and thus reduced below-ground competition (Homulle et al., 2022). Indeed, intercropping has been shown to increase water-use efficiency compared to monocropping practice (Morris & Garrity, 1993). Furthermore, intercropping systems show stable or enhanced crop yield even under drought stress (Renwick et al., 2020). Nevertheless, available evidence in grasslands about a positive relationship between increased water partitioning in more diverse plant communities and increased productivity is controversial with no/limited (Bachmann et al., 2015) but also supporting evidence (Guderle et al., 2018; Mason et al., 2020; O'Keefe et al., 2019; Verheyen et al., 2008). Complementary spatial water uptake patterns of crop species have been described but empirical evidence about water partitioning in intercropping is lacking despite the fact that water partitioning might be one of the mechanisms underlying the often-observed overyielding in mixtures.

The aim of this study was to investigate spatial water uptake patterns and water partitioning of different crop species and how they change with the planting arrangement in monocultures and different mixtures. In particular, the following research questions were addressed: Do different crops use water spatially differently (question 1)? Do spatial water uptake patterns change in response to different neighbouring species (question 2)? Is niche partitioning stronger in mixtures (question 3)? Does niche partitioning result in higher productivity (question 4)? Does competition intensity explain patterns in spatial water uptake (question 5)? How is overyielding in mixtures related to spatial water uptake patterns (question 6)?

A field experiment was conducted where six different crops were grown in different species diversities (one, two or three species grown together). The natural abundance of stable isotopes in plant and soil water and the mixing model MixSIAR (Stock et al., 2018) were used to calculate the proportion of water from different soil layers taken up by the plants grown in different species diversities. Afterwards, niche overlaps of species pairs at each species diversity were calculated by estimating the overlaps from the posterior distribution of the mixing model. These niche overlaps were used as an indicator for niche partitioning, which was then linked to productivity. Finally, the relationship of competition intensity and overyielding in mixtures with spatial water uptake was estimated to test if competition intensity and subsequent changes in spatial water uptake patterns can explain overyielding. We hypothesised that different crop species differ in their spatial water uptake (question 1) and that they plastically respond to species diversity and shift their water uptake patterns when grown with other species (i.e. in mixtures) (question 2). Hence, we expected that niche overlaps in spatial water uptake are lower in mixtures compared to monocultures due to niche differences of the different crop species and phenotypic plasticity in response to interactions with other species (question 3), and that decreased niche overlaps would result in a higher productivity (question 4). Finally, we expected that competition is most intense in shallow soil layers (question 5) and that overyielding is therefore positively related to proportionally more water uptake in deeper soil layers (question 6).

2 | MATERIALS AND METHODS

2.1 | Study site

The field experiment was conducted near Zurich, Switzerland (coordinates 47°26′19.917″N 8°29′58.930″E and 455 m a.s.l.; permission for fieldwork not needed). The field site was located in a temperate climate with annual mean temperature of 9.3°C and annual mean precipitation of around 1000 mm (norm period 1981–2010, MeteoSchweiz). During the field experiment, temperature ranged from 0°C in the beginning of May up to 36°C at the end of June (Figure 1). During June 2019, the hottest days ever were recorded, and June was the second hottest June since 1864. Furthermore, June was a dry month with only two-thirds of usual rainfall during this month (Figure 1).
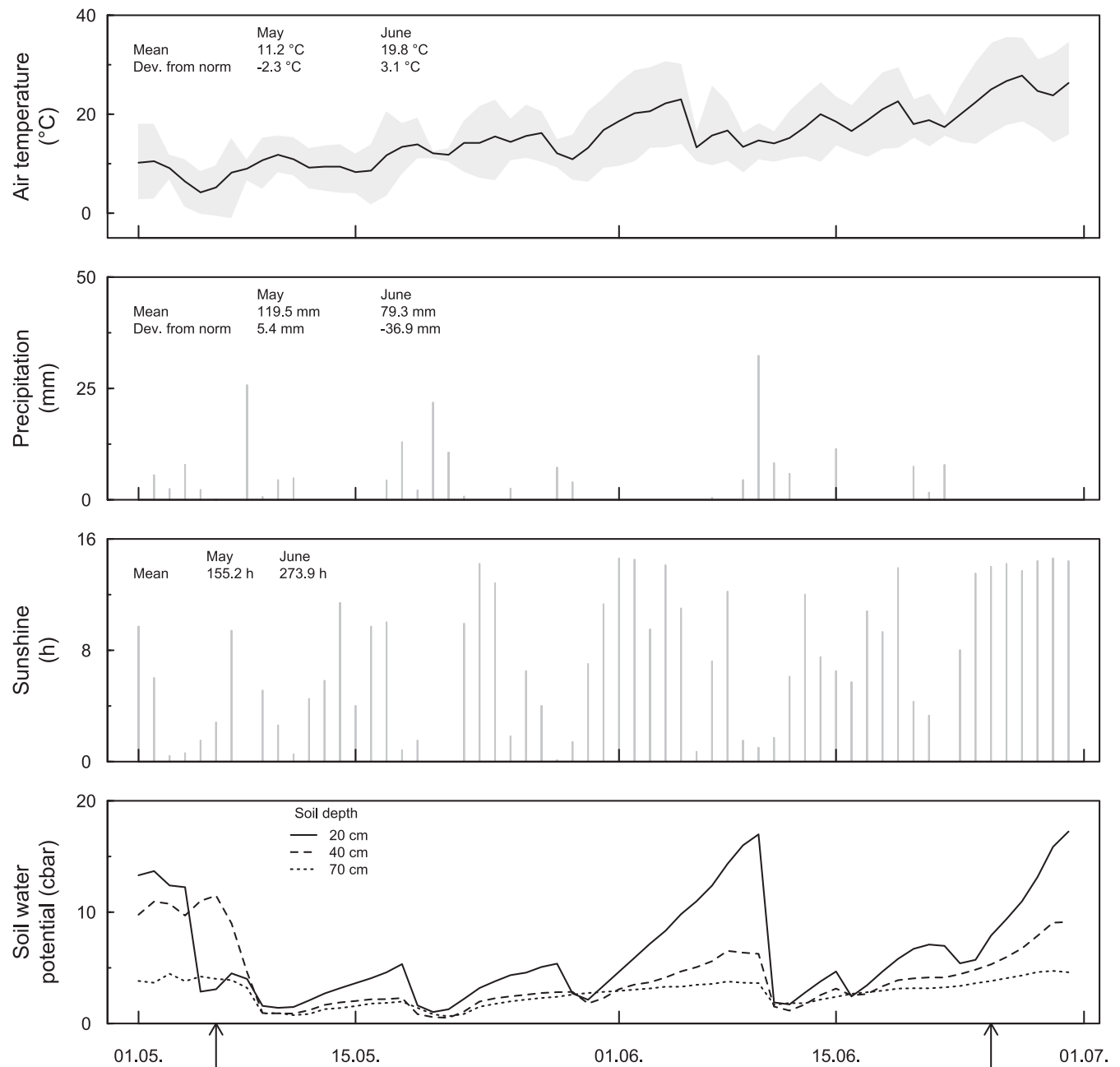
FIGURE 1 Air temperature (day mean ± day minima and maxima), precipitation, sunshine duration and soil water potential between May and June 2019. Arrows indicate sowing (6 May 2019) and the sampling event (25 June 2019). For temperature, the daily mean, minima and maxima are shown. For perception and sunshine duration, the day sum is drawn. The monthly mean of the norm period (1981–2010) and the deviation from the norm period of the months May and June 2019 are also indicated. The data is from the MeteoSchweiz weather station Affoltern and from Kanton Zürich Bodenschutz station in Reckenholz (soil humidity) (approx. 2 km away from the field site).

The soil at the study site was a brown earth and consists of 56% sand, 18% clay, 25% silt, 3% hummus, 8% gravel and 2% stones. Soil water potential in 20 cm depth reflected precipitation, whereas in 70 cm the soil water potential remained steady over time (Figure 1). Soil water potential can roughly be classified in the following groups: saturated-very wet (0–6 cbar), wet-moist (6–10 cbar), moist-dry (10–20 cbar), dry (20–50 cbar) and very dry (>50 cbar). During the duration of the experiment (from sowing until sample collection) the soil in 20 cm depth was wet and only rarely drier (peak in the first part

of June). This indicates that there was no water limitation during the experiment.

2.2 | Seed material

In this experiment, six crop species were used, namely spring barley (*Hordeum vulgare* var. Atrika), spring wheat (*Triticum aestivum* var. Fiorina), faba bean (*Vicia faba* var. Fanfare), pea (*Pisum sativum* var.

Astronaute), linseed (*Linum usitatissimum* var. Marquise) and rapeseed (*Brassica napus* subsp. *Napus* var. Campino). The seeds were purchased from a local retailer (UFA Samen) and are local varieties commonly cultivated in Switzerland. These crop species can be grouped into three functional groups: cereals including wheat and barley, legumes containing faba bean and pea and (oilseed) herbs with linseed and rapeseed. This six crop species have distinct rooting patterns especially with regard to their vertical root distribution (maximal rooting depths are for barley 107 cm, wheat 179 cm, faba bean 90 cm, pea 110 cm, linseed 60 cm and rapeseed 130 cm; from Kutschera et al., 2009).

2.3 | Experimental design

The six crop species were grown in plant communities as monocultures and mixtures with either two or three species. For the 2-species mixtures, all possible combinations among the crop species were cultivated. As for the 3-species mixtures, all possible combinations of crop species between the functional groups were grown together (Table S1). In total 29 different plant communities were grown. The 29 community plots together with three control plots with no plants were arranged in blocks of 4 × 8 plots. This complete set was replicated three times following a randomised-complete block design. Additionally, all crop species were grown as single plants. These single plants experienced no above- and below-ground interaction during the whole cropping season. Thus, these plots were weeded regularly to prevent any growth of weeds which could interact with the single plants. To exclude any above- and below-ground interaction with other crops, all single plants were grown in one separate block which contained five replicates of each species, randomly allocated within the block. All plots measured 0.5 × 0.5 m and were separated by a metal fence to a depth of approx. 30 cm.

Sowing was conducted by hand on the 6 May 2019 with the sowing density recommended by the seed retailer (210 kg ha⁻¹ for wheat, 180 kg ha⁻¹ for barley, 250 kg ha⁻¹ for faba bean, 275 kg ha⁻¹ for pea, 60 kg ha⁻¹ for linseed and 6 kg ha⁻¹ for rapeseed). The sowing ratio (%) was 50:50 and 33:33:33 for 2-species and 3-species mixtures, respectively, except for rapeseed which had 20% in both 2-species and 3-species mixtures (Table S1). Effective plant counts were conducted on the 5 June 2019 in all plots. Since the distinction between barley and wheat in the 2-species mixture was difficult at this early growth stage, the total plant count in these plots was divided by two as the sowing ratio was 50:50. During the whole experiment, the field was neither fertilised nor artificially irrigated (only rainfed, see Figure 1).

2.4 | Water sampling

Sampling for natural ^{18}O and ^{2}H abundance took place on 25 June 2019. In four blocks (three with the community plots and one with single plants), two control plots per block were randomly selected for soil core sampling. Soil cores were taken with a core sampler and the soil

was collected in the following depths: 0, 5, 10, 15, 20, 30, 50 and 75 cm. Given the small scale of the plots and the fact that collecting and characterising soil profiles for each of the 117 plots with plants was not feasible, the empty plots were considered most representative to describe the isotope signal along the soil depth profile, also because they lacked potential crop effects on soil water (e.g. through hydraulic lift).

For the examination of source water, water extracted from the root crown has been shown to match the source water best (Barnard et al., 2006). Hence, for the community plots the root crown of one random individual per species and plot was collected. For linseed, three individuals per plot were pooled, because one individual did not contain enough water for extraction. For the single plants, three plants per crop were randomly chosen for sampling. The soil and plant samples were collected in 12 mL glass vials, closed with a chlorobutyl rubber septum cap (Exetainer©, Labco Limited) and stored at −20°C until further processing.

2.5 | Isotope analysis

Water from samples was extracted following the Cryogenic vacuum method (Dalton, 1988). Subsequently, extracted water was analysed for hydrogen and oxygen isotopic composition (δ^2H and $\delta^{18}O$) following the protocol by Werner and Brand (2001). The results were normalised to VSMOW (Coplen, 1988) and are expressed in the δ-notation (Equation 1).

$$\delta^2H \left[‰ \right] = \left(\frac{\left(\frac{^{18}O}{^{16}O} \right)_{sample}}{\left(\frac{^{18}O}{^{16}O} \right)_{standard}} - 1 \right) * 1000 \tag{1}$$

2.6 | Biomass sampling

During the water sampling, the shoot from the sampled plants were collected in paper bags and dried at 80°C for at least 48 h. Subsequently, the dry biomass was weighed. Initially, it was planned to harvest seed mass after senescence of the plants. However, due to bird and mouse attacks, final seed and biomass data were not available. Hence, the productivity (g/m²) of species grown in community (monoculture, 2- and 3-species mixture) was calculated from the biomass of individual plants collected during water sampling, the number of plants counted after germination and the sowing ratio (productivity (g/m²) = biomass * count / sowing ratio * 4).

2.7 | Data analysis

The complete analysis was carried out in R version 4.0.2 (R Core Team, 2022). For the relations between $\delta^{18}O$ and δ^2H in soil water and the $\delta^{18}O$ and δ^2H along the soil profile linear mixed models were conducted. In the first model, δ^2H was the response variable, $\delta^{18}O$ the predictor and the plot the random term. For the analysis of $\delta^{18}O$ and δ^2H along the soil profile, soil depth was the response, either

$\delta^{18}O$ or δ^2H (second-order polynomial regressions) were the predictors and plot was the random term.

To quantify the proportion of water uptake (PWU) of the different plants grown in the different species diversities (question 1), the Bayesian isotope mixing model MixSIAR (Stock et al., 2018) was used. This model allows to calculate the proportion of sources (here the soil water from a specific soil depth) of unknown mixtures (here the sampled plant xylem water). In advance, the measured $\delta^{18}O$ and δ^2H values from the specific soil depths were combined into the following soil layers: shallow (0–10 cm), middle (15–30 cm) and deep (>50 cm). This spatial stratification of the source allows a better model inference and analysis (Phillips et al., 2005; Stock et al., 2018). Differences between the soil layers were tested with student's t-tests that compare the three soil layers in all possible combinations in both $\delta^{18}O$ and δ^2H values. With MixSIAR, two models were computed. Model 1 describes the PWU from the six crops in the different species diversity levels (the interaction between species and species diversity as fixed factor, specific species composition as random factor; Figure 3). Model 2 was run for each species grown in the specific species composition (specific species composition as fixed factor, no random factor). This model describes how the water uptake differs when specific species are grown together (Figure S1). Each model was run with a chain length of 300′000, burn-in of 200′000, thinning of 100 and with three chains ("long" run in MixSIAR).

Furthermore, niche overlap (as an indicator or niche partitioning) in spatial water uptake was calculated from the overlap of the posterior distribution estimated in model 1 (Figure S2). For this, the overall of the kernel density estimates between all the possible hypothetical crop species pairs of all the different species diversities and soil layers was calculated with the R package OVERLAPPING version 1.6 (Pastore, 2018). The use of overlapping estimates of two or more kernel densities has been suggested for calculating niche overlap rather than only compare means or use the geometrical overlap of kernel densities (Pastore, 2018; Pastore & Calcagnì, 2019; Swanson et al., 2015). Additionally, niche width for all possible hypothetical crop species pairs of all the different species diversities and soil layers was calculated (same as for niche overlap). The niche width was defined by the smallest and largest value of PWU of the species pairs (Figure S2) and is expressed as a proportion of the full range (values between 0 and 1).

In this study, hypothetical species pairs rather can "actual" species compositions were used to be able to calculate niche overlaps not only in the 2-species mixtures but also in the monocultures and 3-species mixtures with the same (hypothetical) species pairs. This allowed to compare expected niche overlaps of any two species (calculated in the monocultures) with observed niche overlaps (calculated in the mixtures) and investigate plasticity of crop species upon interaction with other species.

To investigate the plasticity of spatial water uptake in response to species diversity (question 2), a hierarchical cluster analysis (*hclust* function in R) was applied to the overlapping values. This analysis revealed patterns in niche overlaps in the different species diversity levels, soil layers and hypothetical species pairs. Subsequently, to assess niche overlap in response to species diversity (question 3), the

sums of niche overlaps/niche widths across all three soil layers for each species pair were estimated and paired *t*-tests between species diversities were computed (species pair was the grouping variable).

To calculate the productivity of species pairs, the mean productivity for each species in each species diversity was calculated first and the sum of the estimated productivity for each hypothetical species pair in the separate species diversity levels was calculated and paired *t*-tests were computed (same as for niche overlaps/niche widths). Productivity was first averaged across the species compositions to allow a correlation with estimated niche overlap (which was also estimated for each species at each species diversity level). To test for a relationship between niche overlap and productivity (question 4), correlations between niche overlaps/niche widths and productivity of each species pair were computed for the three species diversities separately. This analysis gives information about the relationship between productivity and niche overlap/niche width of hypothetical species pairs at different species diversity levels.

Furthermore, as a measure of intensity of plant–plant interactions, the relative interaction index (RII) with the biomass of the plant when grown in community (B_c; monoculture, 2- or 3-species mixture, respectively) and when grown without any other plant (B_s; single plants) was calculated for each species in each plot (Armas et al., 2004; Equation 2). The RII gives information about how the biomass changes upon interaction with neighbours, RII < 0 indicates competition (negative interaction) whereas RII > 0 indicates facilitation (positive interaction). RII was previously transformed to allow for square-root transformation ($RII_t = (RII + 1)/2$). Subsequently, a LMM was computed to test if RII for the different crop species differed depending on species diversity. The square-root transformed RII_t for each species and each plot was the response variable, the block, diversity (monoculture vs mixture) and the mixture diversity (2- vs 3-species mixture) nested in diversity were the explanatory variables and the species composition was set as random term.

$$RII = \frac{(B_c - B_s)}{(B_c + B_s)}. \tag{2}$$

Finally, the partial land equivalent ratio (PLER) for each species in each mixture diversity (2- and 3-species mixture) and species composition was calculated. The PLER was defined by the productivity in the mixture divided by the mean productivity in the monoculture ($PLER = P_{mix}/P_{mono}$). PLER gives information about species-level overyielding in mixtures in comparison to the corresponding monocultures.

For the relationship between RII/PLER and mean PWU estimated in model 2 (question 5 and 6), generalised linear mixed models (GLMMs) with beta distribution were computed. Prior, mean RII of the three replicates were calculated. Since the mean PWU of the three soil layers are not independent, a separate model for each soil layer was implemented. The mean PWU was the response variable; RII and PLER, respectively, species and diversity were the explanatory variables; the species composition was set as random term. Estimated marginal means (aka least-squares means) were used to estimate the regression lines. To test for a correlation between PLER

and RII in plants grown in mixture, a linear model was performed with PLER as response variable and RII, species and mixture diversity as explanatory variables.

3 | RESULTS

3.1 | Isotopic signature of soil water

The $\delta^{18}O$ and δ^2H values from water extracted in the specific soil depths were correlated ($p < 0.001$) and showed $\delta^{18}O$ values between −1‰ and −11‰ and δ^2H values between −27‰ and −77‰ (Figure 2a). The isotopic signature along the soil profile showed the following pattern: The topsoil was isotopically enriched, and the isotopic signatures of $\delta^{18}O$ and δ^2H decreased with increasing soil depth (Figure 2b,c, Table S2). The different soil layers (shallow, middle and deep) showed significantly different isotopic signatures in both $\delta^{18}O$ and δ^2H ($p < 0.001$ between all soil layers, data not shown).

3.2 | Proportion of water uptake in the different soil layers

To address question 1, mixing models were used to estimate variations in spatial water uptake of crop species grown in different species diversities. The results suggested that most of the water was taken up in the shallow soil layer (Figure 3). The importance of the middle soil layer (15–30 cm) varied mostly between the species but also within the species between diversities (Figure 3). In general, the deep soil layer (>50 cm) was not an important water source at the time of measurement. For the cereals, barley and wheat, the shallow and middle soil layers were both important sources of water. For wheat, the proportion remained unchanged across all diversities whereas in barley a trend towards a higher PWU in the shallow soil layer with increasing species diversity was found. For both legumes, faba bean and pea, most of the water was taken up in the shallow soil layer and this did not change among the diversity levels. For linseed, shallow and middle soil layers were both an important source of water. On the other hand, rapeseed showed a clear shift from shallow to middle soil layer, when grown in mixtures compared to when grown as single plant or in monoculture.

3.3 | Niche overlap in spatial water uptake

The second analysis aimed to investigate if spatial water uptake patterns changed in response to varying intercropping species (question 2). The cluster analysis revealed three distinct clusters of PWU among the different diversity levels and soil layers (Figure 4, x-axis): the first cluster with the monocultures independent of soil layer (D1) and the deep soil layer independent of the diversity level (L3), the second cluster with the shallow and middle soil layer (L1 and L2) in single plants (Ds) and the third cluster with the shallow and middle soil layer (L1 and L2) in 2- and 3-species
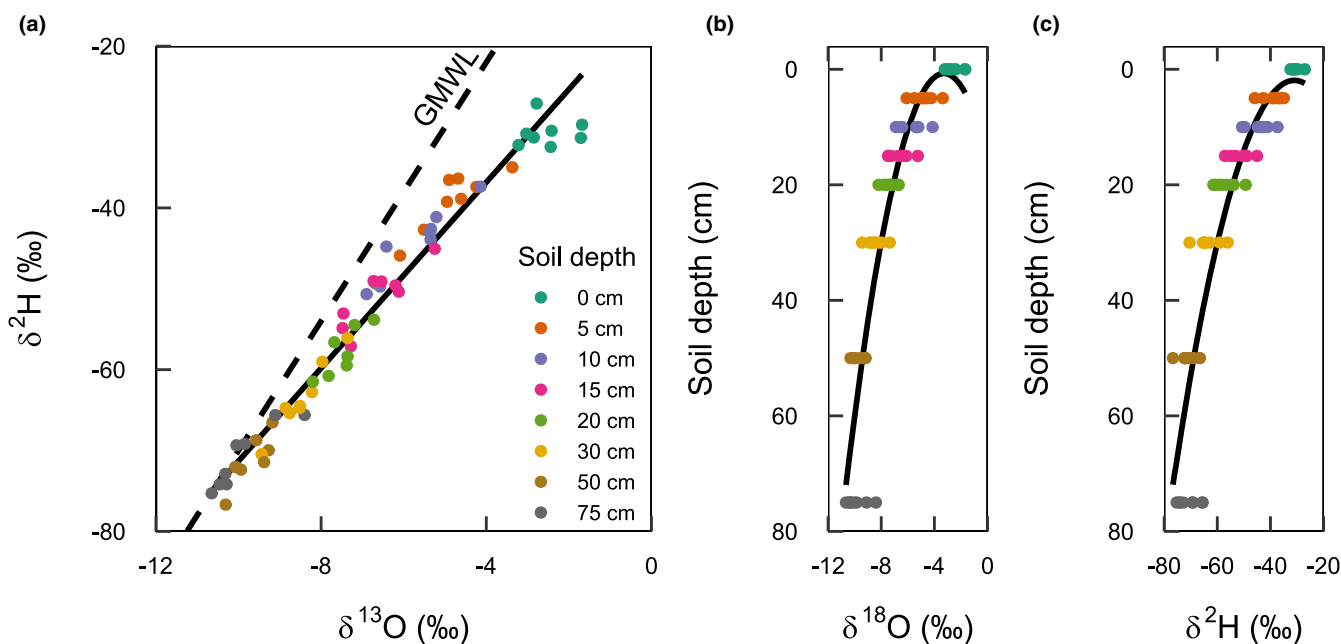

FIGURE 2 Isotopic signature of $\delta^{18}O$ and δ^2H (‰) from water extracted at specific soil depths. (a) Relationship between $\delta^{18}O$ and δ^2H. Water extracted from the soil depth are coloured accordingly (points in different colours). The regression line of the soil water is shown (solid line; $y = −13.8 + 5.7x$; $p < 0.001$; $N = 64$). The global meteoric water line is also shown (GMWL; dashed line; $y = 10 + 8x$). (b) δ^2H values along the soil profile. Shown is the regression line ($y = −26 + 165 − 48x^2$; $p < 0.001$) and the individual samples per soil depth (coloured points; $N = 8$). (c) $\delta^{18}O$ values long the soil profile. Shown is the regression line ($y = −26 + 162x − 65x^2$; $p < 0.001$) and the individual samples per soil depth (coloured points; $N = 8$).
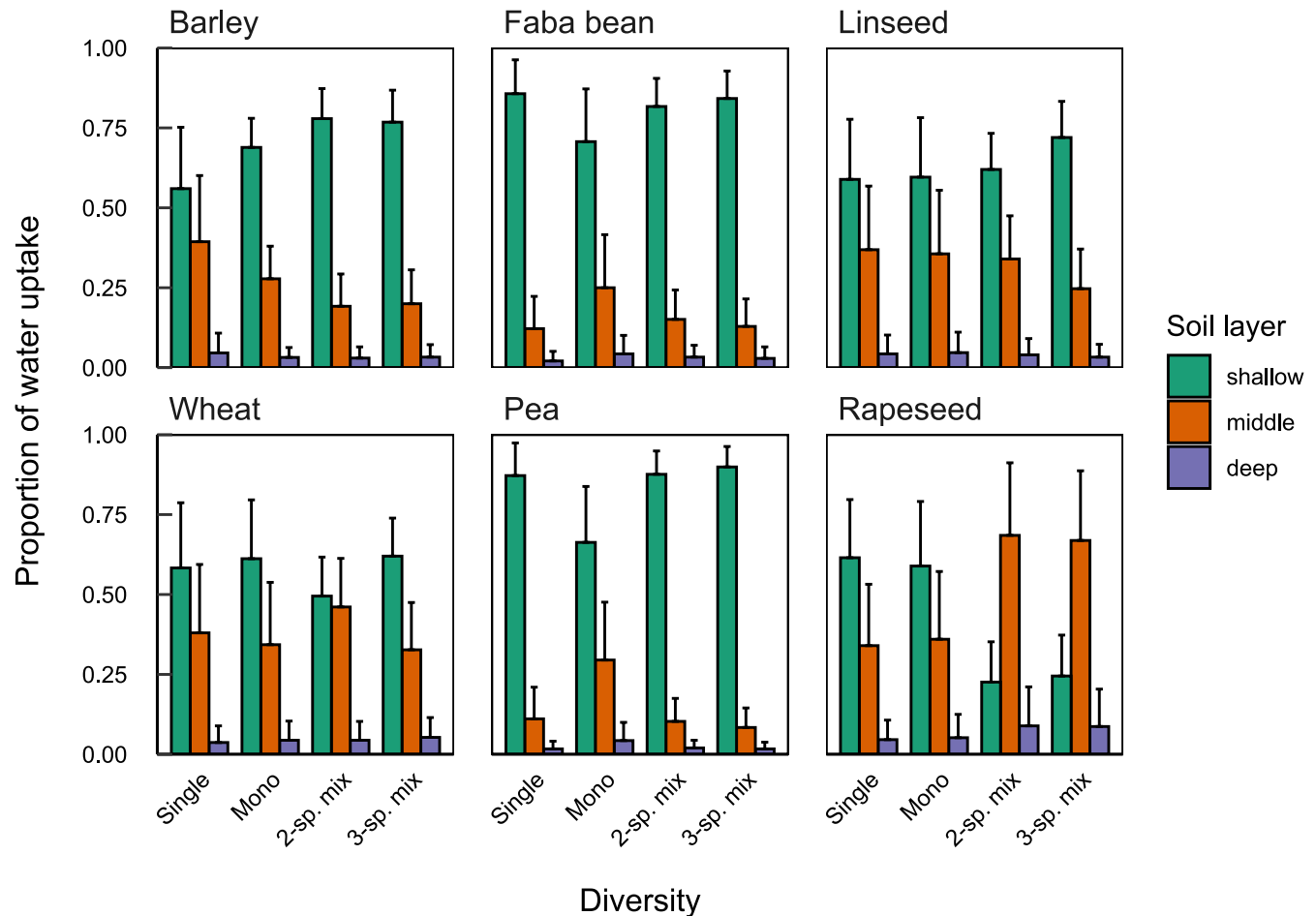
FIGURE 3 Proportion of water uptake from the different soil layers by the six crops barley, wheat, faba bean, pea, linseed and rapeseed grown as single plant, in monoculture and in 2- and 3-species mixtures (mean $\pm$ standard deviation of the mean). The proportions were calculated with the mixing model MixSIAR (model 1; species*diversity as fixed factor, species composition as random factor).

mixtures (D2 and D3). The first group including all monocultures was characterised with high overlap values (≥0.5). This means that monocultures of any species tended to distribute roots similarly. In the second cluster including the single plants, overlap values depended strongly on the hypothetical species combination. This indicates that when grown without competition some species (especially the two legumes) had more distinct root distributions than the other species. When the two legumes (faba bean and pea) would be combined with the other species, overlap values would be low (<0.3) whereas these values would be higher with combinations among the other species (>0.8). The third cluster including all the mixtures showed low to very low overlap values (<0.5, with some exceptions). This indicates that overlap in root distribution was generally lower in mixtures than monocultures.

The clustering of the species combinations revealed two clear clusters (Figure 4, *y*-axis). These two clusters mainly differed in their overlap value estimated for the two mixtures (D2 and D3) in the two upper soil layers (L1 and L2). The cluster with higher overlap values included all mixtures with barley or faba bean, except when they were combined with rapeseed and faba bean-wheat,

and the combination wheat-linseed. Overlap values of rapeseed with any other species were low (<0.29), as were the combinations of wheat with faba bean and pea, and pea-linseed. To summarise, niche overlap in water uptake was lowest in the mixtures—especially when the combination included rapeseed or wheat with legumes.

3.4 | Linking niche overlap and niche width to productivity

Niche overlaps between species pairs were related to species diversity and productivity (Figure 5). Productivity of hypothetical species pairs was significantly higher in the 2- and 3-species mixtures compared to monocultures (Figure 5a). Analogous, the sums of niche overlaps and niche widths across all soil layers per plot were significantly higher in monocultures than mixtures (Figure 5b,c). Furthermore, there was no relationship between niche overlap/niche width and productivity in any species diversity (Figure S3).
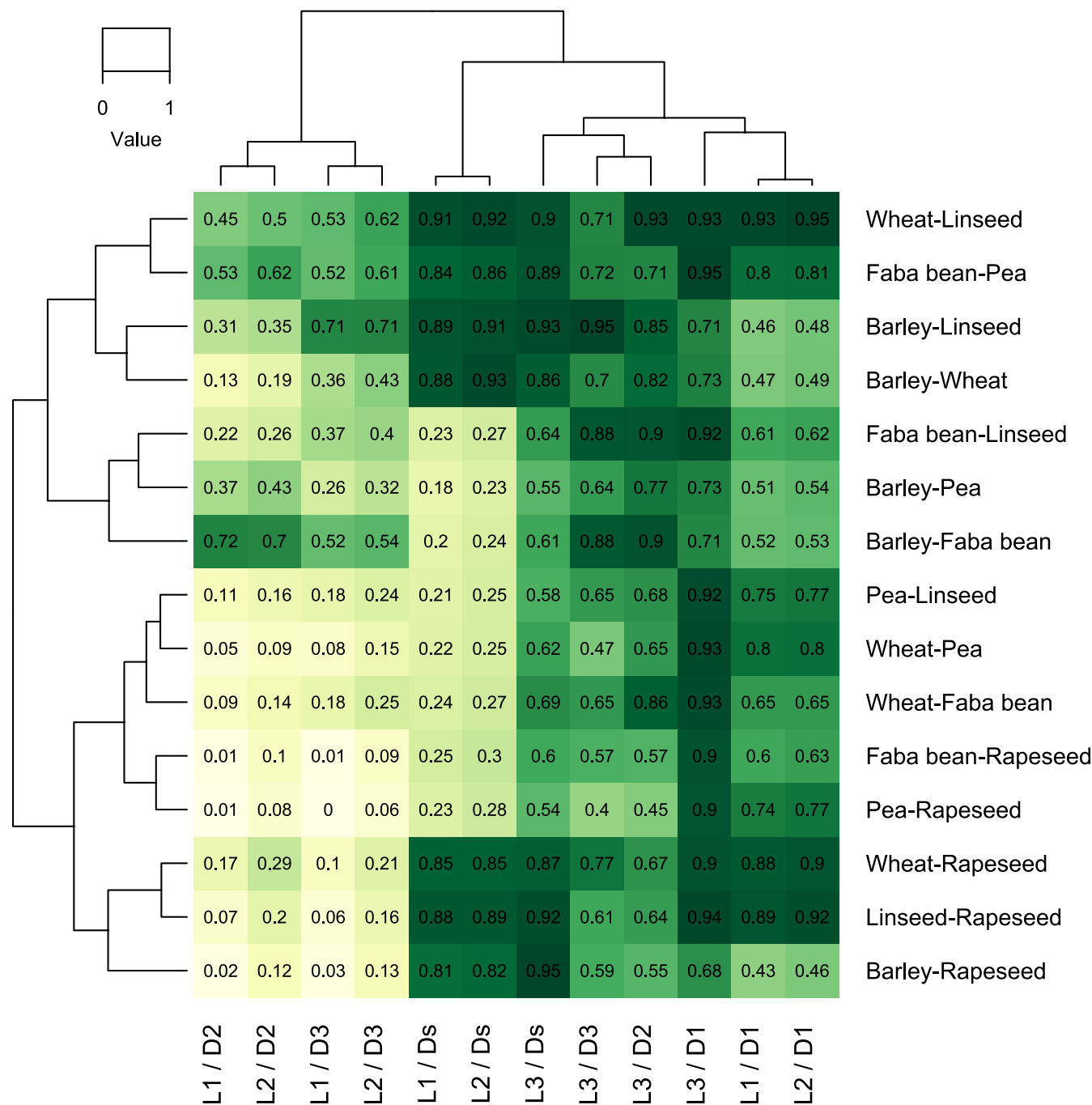
FIGURE 4 Heatmap of the estimated overlaps from the posterior densities estimated by model 1. Overlaps were calculated between all the possible combinations of species and for the interactions of soil layer (L; 1 = shallow, 2 = middle, 3 = deep) and the diversity (D; s = single plant, 1 = monoculture, 2 = 2-species mixture, 3 = 3-species mixture). Overlap values range from zero to one and are also indicated in the corresponding squares. Histograms show hierarchical clustering (*hclust* function in R).

3.5 | Relationship of spatial water uptake with competition intensity and overyielding

RII differed among species (Figure 6). Rapeseed showed the highest reduction of biomass upon interaction with neighbours (approx. −0.85), followed by barley (approx. −0.7), wheat and linseed (both approx. −0.6). Competition was relatively weak for the two legumes faba bean and pea. Nonetheless, the RII, as it was the

case for all species, was significantly smaller than zero ($p < 0.001$, Table S3). Regarding species diversity, only linseed showed a response to increasing crop diversity with lower RII (higher competition) in the two mixtures compared to the monoculture ($p = 0.047$, Table S3).

In order to investigate whether patterns in water uptake change upon interaction intensity (question 5), the relationship between mean PWU and RII was estimated. The GLMM and
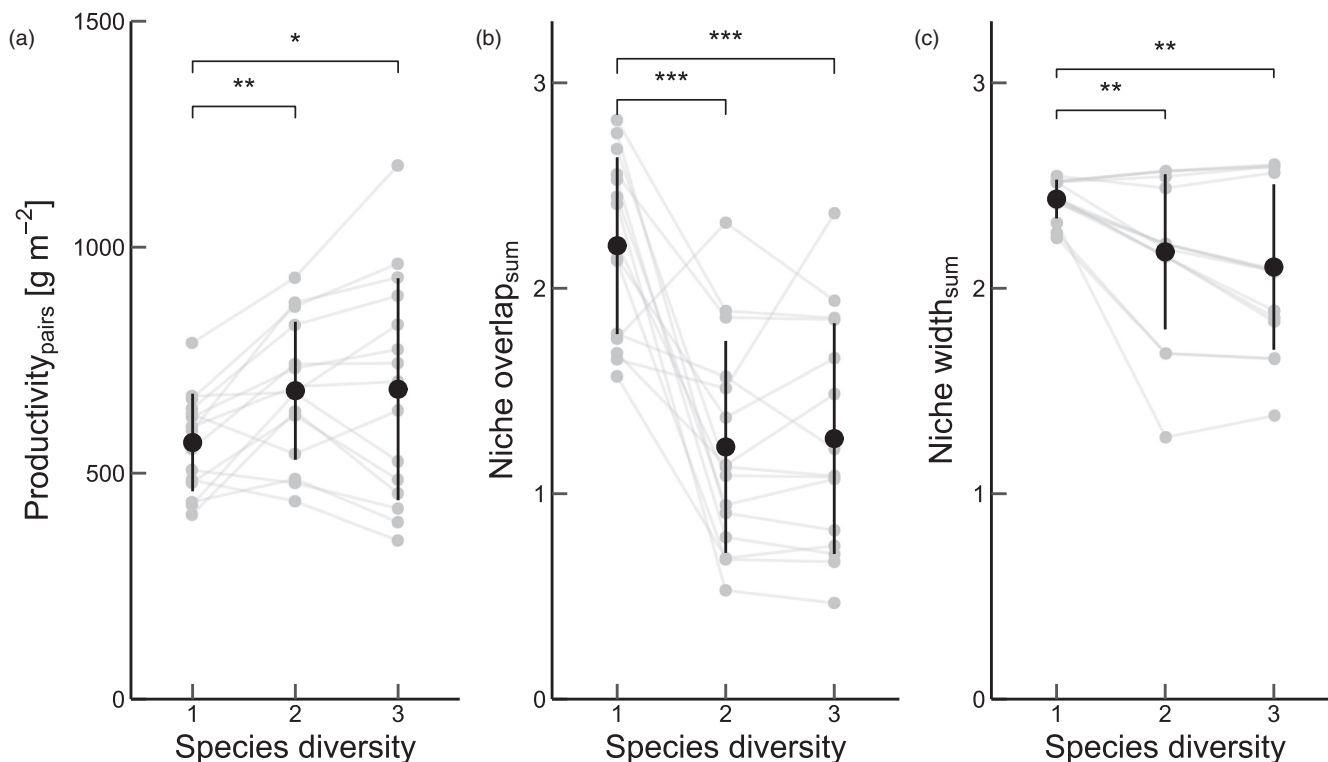
FIGURE 5 (a) Productivity, (b) niche overlap (sum) and (c) niche width (sum) of hypothetical species pairs grown in different species diversities. Shown are the single data points (grey points), the connection between paired data points (hypothetical species pairs; grey lines) and the mean ± standard deviation of the mean (black point and line). Significant differences of a paired t-test are indicated with asterisk and brackets. Significance code corresponds to *: $p < 0.05$, **: $p <= 0.01$, ***: $p <= 0.001$.

subsequent ANCOVA indicated significant effects of RII on mean PWU in all soil layers (Figure 7, Table S4). This means that plants which experienced intense competition (lower RII values) used more water from shallow soil layers. With decreasing competition intensity (higher RII values) the middle and deep soil layer became more important, and the shallow soil layer declined in importance to a point where shallow and middle soil layers were an equally abundant water source.

With regard to the relationship between PLER and mean PWU in mixtures (question 6), PLER had a significant effect on mean PWU in all soil layers (Figure 7, Table S5). The trends between the soil layers appeared the same as for RII: With higher PLER, the mean PWU decreased in the shallow and increased in the middle and deep soil layers. Indeed, an ANCOVA revealed that the two variables RII and PLER were positively correlated (Figure S4, Table S6).

4 | DISCUSSION

4.1 | Isotopic signature along the soil profile and soil water potential

The soil profile in this study showed clear enrichment in heavy isotopes (i.e. higher $\delta^{18}O$ and δ^2H) in the top soil and a continuous decrease with soil depth (Figure 2). Furthermore, when comparing the

global meteoric water line, the collected stable isotopes in this study are less enriched in δ^2H compared to $\delta^{18}O$. This effect is due to evaporation (Cappa et al., 2003) in the top soil. Temperature, precipitation, and soil water potential data support these findings (Figure 1). Since the last rain event in the first part of June, topsoil (20 cm) was continuously drying out—but only to a point where the soil can be classified as moist at the time of data sampling.

It is important to note that no soil moisture data is available for the study site directly or the different plots which could explain patterns in spatial water uptake. However, the plots are arranged on a small scale and thus diffusion between plots cannot be excluded. Hence, large differences in soil moisture distribution between species diversities and/or species compositions are not expected.

4.2 | General patterns in spatial water uptake and the relationship to root distribution

The shallow (0–10 cm) and middle (15–30 cm) soil layers were the most relevant water sources for the inspected crop species (Figure 3). These findings are in accordance with the plant water uptake globally in the temperate climate zone (Amin et al., 2019) and grassland species that were grown in different diversities (Guderle et al., 2018). Nevertheless, spatial water uptake depends strongly on plant growth stage (Ma & Song, 2016, 2018; Wang et al., 2017;
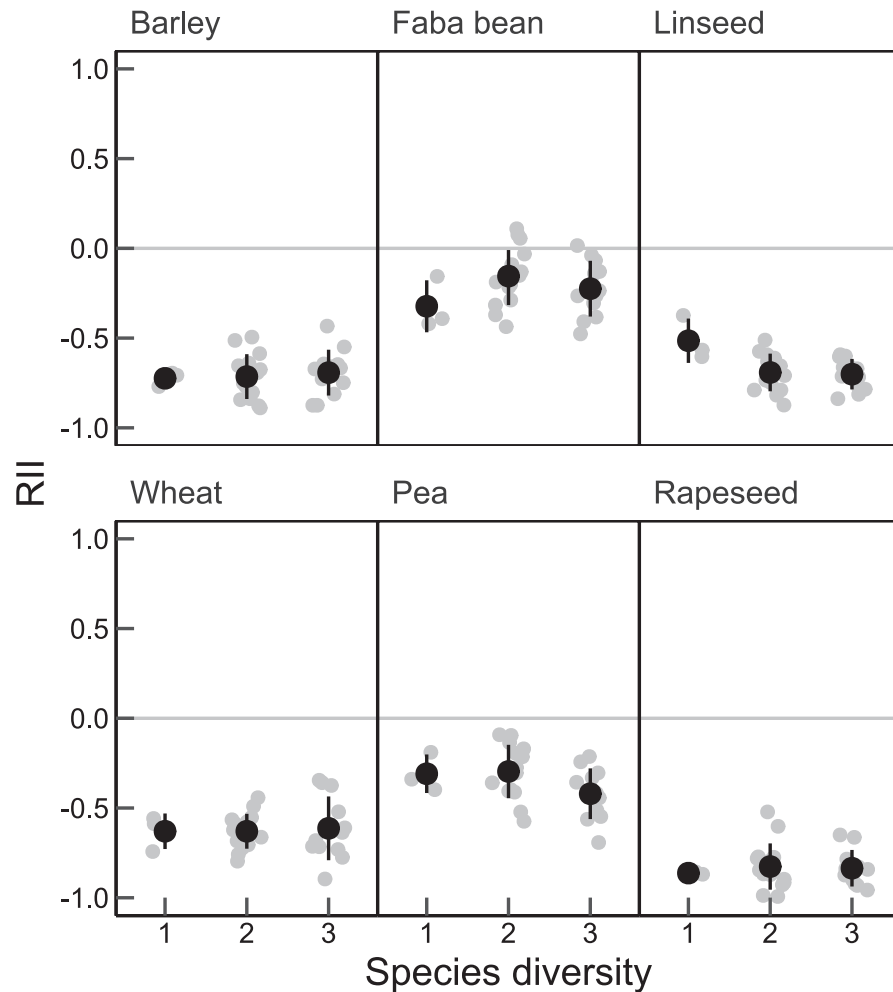
FIGURE 6 Relative interaction index (RII) calculated as the relative change in biomass between individuals grown in community (monocultures and 2- and 3-species mixtures) and as single plant without neighbours. Drawn is the mean ± the standard deviation of the mean (black points and lines) and the individual data points (grey points).
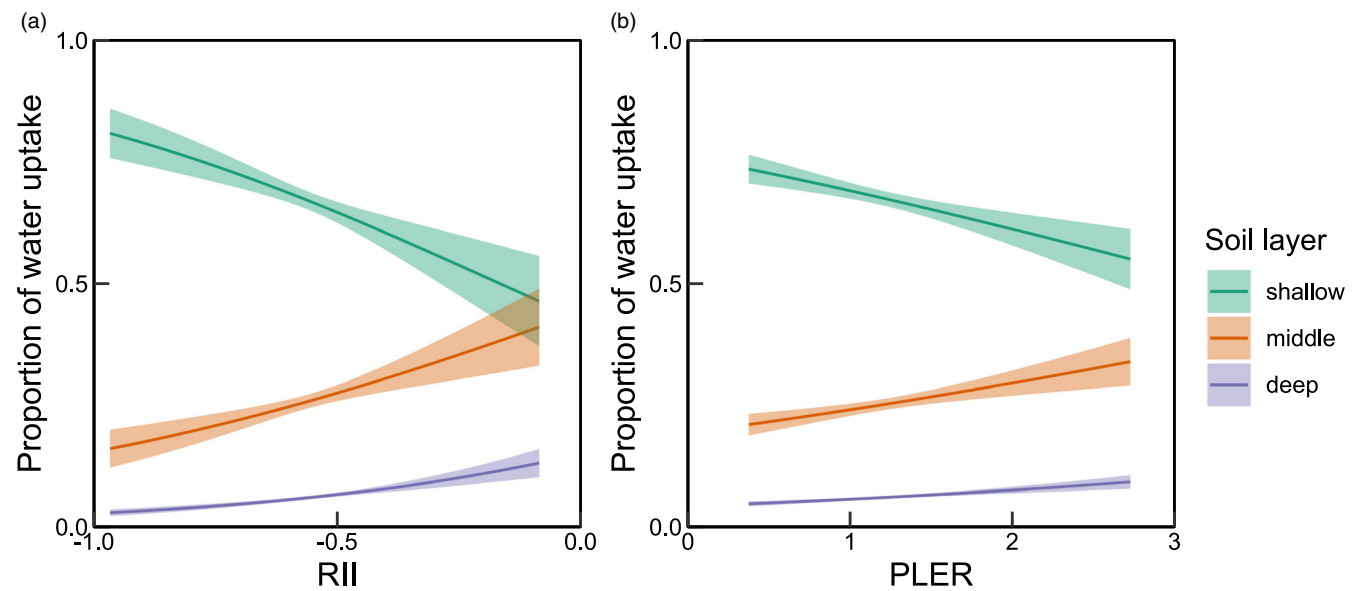

FIGURE 7 Relationship of (a) relative interaction index (RII) and (b) partial land equivalent ratio (PLER) with proportion of water uptake (from model 2) in the different soil layers (shallow, middle and deep). Shown are the regression lines of the generalised linear mixed models.

Wu et al., 2018). These studies showed that crop species used a big proportion from shallow soil (0–20 cm) in early stages and shifted to deeper soil layers just before flowering. In our study, the crops were in the stage of heading (wheat and barley), flowering (faba bean) and early fruit development (pea, linseed and rapeseed). During these stages, the roots are expected to be fully developed (Weaver, 1926).

Plant roots therefore explored the greatest possible soil area and depth.

The six different crop species used in this study have distinct rooting patterns (Fan et al., 2016; Kutschera et al., 2009). For instance, rapeseed has a higher root length density compared to other oilseed and pulse crops (among others linseed and pea; Liu et al., 2011), whereas faba bean has in general a shallow root system (Li et al., 2006). These distinct rooting patterns are reflected in the observed differences in spatial water uptake of the crop species when grown as single plants. Furthermore, due to the gradient of water isotopes along the soil profile (Figure 2), water isotopes can be used to indirectly explain root distribution especially when comparing between different species diversities. This might again be relevant for nutrient uptake (Andresen et al., 2016; Barley, 1970). Nevertheless, there is still a lack of research about spatial root distribution and resource uptake and how different resource uptake patterns are correlated.

4.3 | The effect of crop diversity on resource partitioning and plasticity in water uptake

Literature suggests that resource partitioning is stronger in more diverse communities due to differences in resource uptake between species (Barry et al., 2019). For example, when species are grown in monoculture, the whole community uses the same resources. However, when two or more species are grown together, species differ in resource uptake and, hence, available resource uptake by the community can be exploited more efficiently. On the other hand, results from field experiments suggest plasticity in spatial water uptake in response to species diversity in grassland communities (Guderle et al., 2018). This might be due to plant root plasticity in response to neighbouring plants (Callaway et al., 2003) where plants segregate roots spatially to avoid competition (Schenk et al., 1999). With regard to crop species, domestication of crops might have reduced the plasticity, for example in water use (Matesanz & Milla, 2018)—even though plants are assumed to be highly plastic in general (Sultan, 1987).

Results in this study suggest plasticity of water uptake in response to species diversity (Figure 4). Niche overlaps of hypothetical species pairs were reduced in the mixtures compared to the niche overlaps expected based on monocultures (Figure 5). This indicates shifts to different water sources when grown with other species. Particularly, hypothetical species pairs with wheat or rapeseed in mixtures showed the strongest reduction in niche overlaps. Nonetheless, plasticity does not only decrease niche overlaps (Lipowsky et al., 2015). In our system, however, only a few hypothetical species pairs showed increased niche overlaps (e.g. barley-faba bean in 2-species mixture and barely-linseed in 3-species mixture). Additionally, literature suggests that both selection and plasticity are important for niche partitioning (Meilhac et al., 2020). Since seeds in this study originated from a commercial seed supplier, crop species did not experience prior selection

to, for example, mixed cropping. Thus, observed differences between monocultures and mixtures were mainly driven by plasticity (Figure 5). For crop production, plasticity might be relevant (Nicotra & Davidson, 2010) especially for resource partitioning in mixtures (Zhu et al., 2015). It is proposed that future breeding programs should emphasise on adaptive plasticity of crop species (Brooker et al., 2022; Milla et al., 2017) such as root plasticity (Schneider & Lynch, 2020) especially in more variable environments and due to climate change (Matesanz et al., 2010; Nicotra et al., 2010).

4.4 | Relationship between resource partitioning and productivity

Increased resource partitioning in mixtures can explain why mixtures perform better than monocultures (Mason et al., 2020; Verheyen et al., 2008). In this study, we found lower niche overlaps and higher productivity in mixtures compared to monocultures (Figure 5). Nevertheless, no relationship was present between niche overlaps and productivity in the different species diversities (Figure S3). Together with the assumption that reduced niche overlaps indicate enhanced water partitioning, these results suggest that spatial water partitioning was not the (main) driver that led to higher productivity. Temporal, spatial or chemical partitioning of other resources such as nitrogen (Ashton et al., 2010; Engbersen et al., 2021) or light (Engbersen et al., 2022; Mason et al., 2020) could also explain higher productivity in mixtures. In contrast, other studies suggest that below-ground resource uptake might not explain the positive relationship between species diversity and productivity (Jesch et al., 2018; von Felten et al., 2009). Another explanation could be that the measured spatial water uptake at a specific time point cannot explain higher productivity. Furthermore, productivity was measured early in the cropping season (during flowering), and hence effects of water partitioning might not yet have been apparent. This is also a limitation of this study: Looking at only one time point might not be enough to study the impact of resource uptake on productivity (Trinder et al., 2013). A method which integrates both spatial and temporal water uptake might shed more light on the relevance of resource partitioning (Jesch et al., 2018).

4.5 | Competition intensity, overyielding and spatial water uptake

In our system, competition (i.e. RII) was very strong (Figure 6). All species showed a reduction of biomass when grown in communities. However, literature suggests that more diverse communities show reduced competition due to niche partitioning (Loreau & Hector, 2001). In our system, the effect of crop diversity on competition intensity was limited, and even showed the opposite direction (stronger competition with increasing crop diversity as seen in

linseed). Competition and spatial water uptake also showed a significant relationship: upon more intense competition, crop species used more water from shallow soil and less from deeper soil (Figure 7). This means competition was more intense in the shallow than the deep soil layer and that less competitive plants were pushed into deeper soil layers whereas more competitive plants were able to remain in shallow soil. In a similar vein, enhanced overyielding in mixtures (PLER > 1) was related to gradually increased water uptake in deeper soil layers (and gradually less uptake in the shallow soil layer).

4.6 | Conclusion and further research

The aim of this study was to investigate spatial water uptake patterns in crop species and how these patterns change with crop diversity. Furthermore, niche overlaps in spatial water uptake were used to assess niche partitioning between species pairs. The applied methods here allowed to evaluate how expected niche overlaps (calculated in the monocultures) change upon interaction with other species (calculated in the mixtures). This provided insight about the mechanisms behind water partitioning in mixtures (i.e. plasticity vs inherent differences between species). With this method, we were able to show that crop species in our study were highly plastic and shifted to other water sources when grown with other species. Furthermore, we showed that water partitioning and productivity were increased in mixtures compared to monocultures. Nonetheless, water partitioning did not (fully) explain higher productivity in mixtures since we did not find a correlation between the two variables. Furthermore, we showed that competition was more intense in the shallow than in deeper soil layers and that overyielding in mixtures was driven by the extended uptake of water from deeper soil layers. As far as we know, no other study empirically investigated this link between overyielding in mixtures and spatial water uptake patterns.

It would now be interesting to investigate different irrigation and/or drought scenarios to test water partitioning in the different diversities and with different water availabilities. This might shed more light on drought resilience of crop mixtures—which would be relevant especially in rainfed agricultural areas and where drought events might become more frequent due to climate change (IPCC, 2014).

AUTHOR CONTRIBUTIONS

Anja Schmutz planned and conducted the experiment, analysed the data and wrote the manuscript. Christian Schöb gave inputs to the experimental design, data analysis and the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/1365-2745.14088.

DATA AVAILABILITY STATEMENT

The data used in this study is available on Zenodo https://doi.org/10.5281/zenodo.7505603 (Schmutz & Schöb, 2023).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Overview of the different diversity levels (community, diversity, and mixture diversity), the species combinations and the sowing ratios.

Figure S1. Proportion of water uptake from the different soil layers by the six crops barley, wheat, faba bean, pea, linseed and rapeseed grown in 2- and 3-species mixtures (mean ± standard deviation of the mean). The proportions were calculated with the mixing model MixSIAR (species*diversity*species composition as fixed factor, no random factor).

Figure S2. Kernel densities from posterior distributions of water uptake proportions estimated by the mixing model MixSIAR (model 1; species*diversity as fixed factor, species composition as random factor) in the different soil layers (shallow, middle and deep) and in the different diversity levels (single plant, monoculture and 2- and 3-species mixtures) for the six species (coloured lines). Note the different y-axis scales.

Figure S3. Relationship between productivity and (a) niche overlap and (b) niche width of hypothetical species pairs grown in monocultures (blue) and 2- (yellow) and 3-species mixtures (red). The regression line and the results from the Pearson correlation are shown.

Table S2. Type I-analysis of variance of the linear mixed models in Fig. 2. The first LMM (relationship between $\delta^{18}O$ and δ^2H) the response variable was $\delta^{18}O$, the predictor δ^2H and the random term the plot. In the LMM of $\delta^{18}O$ and δ^2H along the soil profile, respectively, the response was the soil depth, the predictors $\delta^{18}O$ and δ^2H, respectively, and the random term the plot.

Table S3. Type III-analysis of variance of the linear mixed model with the square-root transformed relative interaction index (RII) as response variable and block, diversity (monoculture vs mixture) and mixture diversity (2- vs 3-sepcies mixture) as explanatory variable and species composition as random term. Df: degrees of freedom, denDf: denominator degrees of freedom, F: probability distribution, P: error probability. P-values in bold are significant with α=0.05.

Table S4. Type III-analysis of variance of the generalised linear mixed models with the estimated proportions of water uptake (model 2) as response variable (each soil layer separately), the relative interaction index (RII), species and diversity (monoculture, 2- and 3-species mixture) as explanatory variables and species composition as random term. Df: degrees of freedom, Chisq: Chi-square statistic, P: error probability. P-values in bold are significant with α=0.05.

Table S5. Type III-analysis of variance of the generalised linear mixed models with the estimated proportions of water uptake

(model 2) as response variable (each soil layer separately), the partial land equivalent ratio (PLER), species and mixture diversity (2- and 3-species mixture) as explanatory variables and species composition as random term. Df: degrees of freedom, Chisq: Chi-square statistic, P: error probability. P-values in bold are significant with α=0.05.

Figure S4. Relationship between partial land equivalent ratio (PLER) and relative interaction index (RII) in plants grown in mixtures. Shown are the single data points and the regression line (estimate ± standard error) from the linear model.

Table S6. Type I-analysis of variance of the linear models with the land equivalent ratio as response variable and the relative interaction index (RII), species and mixture diversity (2- and 3-species mixtures) as explanatory variables. Df: degrees of freedom, F: probability distribution, P: error probability. P-values in bold are significant with α=0.05.