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Plant water uptake along a diversity gradient provides evidence for complementarity in hydrological niches

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Biodiversity enhances a variety of ecosystem processes, and yet the underlying mechanisms through which these relationships occur remain a critical knowledge gap. Here, we used the natural abundance of stable isotopes to measure depth of water uptake in five common grassland species (*Asclepias tuberosa*, *Lespedeza capitata*, *Liatris aspera*, *Schizachyrium scoparium* and *Sorghastrum nutans*) growing across an experimental grassland diversity gradient. Using this approach, we addressed the following questions: 1) does the depth-specific provenance of water uptake differ among species and/or do interspecific differences in water source manifest with increasing community diversity? 2) Does the isotopic niche space occupied by plants change with increasing diversity? 3) Is plasticity in water uptake depth across a diversity gradient associated with functional plant responses? We found that the depth of soil water used by plants was inherently different among species when grown in monocultures. All species used less shallow soil water and more intermediate-depth soil water in mixed assemblages than in monocultures, resulting in similar interspecific differences in water source across the diversity gradient. However, plasticity in the locations of water used were positively associated with increases in plant growth in higher diversity treatments. These results indicate that plasticity in water-use may contribute to positive biodiversity–productivity relationships commonly observed in temperate grasslands.

Keywords: biodiversity, diversity–productivity relationship, ecophysiology, grasslands, niche complementarity, overyielding, plant water source, stable isotopes, transpiration

Introduction

A substantial body of work has shown that biodiversity enhances ecosystem functioning (Naeem et al. 1994, Hooper et al. 2012, Allan et al. 2013). In terrestrial ecosystems, plant biodiversity increases productivity (Hector et al. 1999, Tilman et al. 2001), alters carbon, water and nutrient fluxes (Dybzinski et al. 2008, Milcu et al. 2016), and improves ecosystem goods and services (Díaz et al. 2005, Isbell et al. 2017b). Diverse plant communities are also more functionally stable through time (Lehman and Tilman 2000, Isbell et al. 2009) and are better able to resist and recover from disturbances

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and extreme climate events (Kreyling et al. 2017, Wagg et al. 2017). These relationships have been observed using a variety of systems including long-term experimental manipulations (Tilman et al. 2001), natural ecosystems (Duffy et al. 2017), and agroecosystems (Isbell et al. 2017a). Considering the widespread occurrence of this phenomenon, as well as the recent documented declines in global biodiversity (Newbold et al. 2015), understanding how biodiversity alters ecosystem processes in mechanistic detail is of critical importance.

Despite the extensive experimental evidence showing a positive biodiversity–ecosystem functioning relationship (Cardinale et al. 2007, Tilman et al. 2012), the mechanisms underlying this phenomenon remain unresolved (Silvertown 2004, Hooper et al. 2005). One commonly proposed explanation is niche complementarity (Loreau and Hector 2001, Fargione et al. 2007), which occurs when coexisting species have unique traits that promote spatial or temporal differences in resource use within a community (Chase and Leibold 2003, Silvertown 2004). Consequently, species within these communities experience less competition (MacArthur and Levins 1967, Silvertown et al. 1999), exploit available resources more completely (Tilman et al. 1996), and are more productive than monocultures (Hector et al. 1999, Tilman et al. 2001). Outperformance of mixtures over monoculture is known as ‘overyielding’ and is typically used as an indirect test for complementarity in biodiversity–ecosystem functioning research (Loreau et al. 2001, Cardinale et al. 2007). However, mixed-species assemblages do not always produce more biomass than monocultures (Cardinale et al. 2011), which may be explained by experimental factors rather than an actual lack of niche complementarity (e.g. statistical limitations in detecting overyielding or insufficient time to demonstrate overyielding; Cardinale et al. 2007). Furthermore, recent modeling efforts have shown that biomass overyielding could result from processes other than resource-use complementarity, such as facilitation (Wright et al. 2017). Thus, niche complementarity is difficult to test in natural and experimental settings, warranting a need for more direct evidence of resource use and niche space in plant communities.

Given that water plays an important role in driving a variety of grassland processes such as primary productivity, plant physiology, microbial activity and ecosystem carbon and water fluxes (Knapp et al. 2001, Huxman et al. 2004a, b, Petrie et al. 2012, Zeglin et al. 2013), hydrological niche complementarity may be an important mechanism contributing to the positive biodiversity–ecosystem functioning relationship in this biome. Hydrological niche complementarity, or niche partitioning of soil water, can occur due to interspecific differences in root distribution (Ward et al. 2013, Li et al. 2018) or via dynamic uptake responses to shifting water availability (Nippert and Knapp 2007, Asbjornsen et al. 2008). Spatial and temporal niche partitioning of soil water has been observed in a variety of natural herbaceous (Nippert and Knapp 2007, Kulmatiski and Beard 2013) and woody (Schume et al. 2003, Schwendenmann et al. 2015) ecosystems worldwide. Interestingly, niche partitioning of water is seldom investigated in biodiversity experiments (but see Bachmann et al. 2015, Schwendenmann et al. 2015), and thus, whether hydrological complementarity directly improves ecosystem functioning is not well established. Whether complementarity, when it is observed, arises from inherent interspecific differences in water uptake or if plants experience plastic shifts in depth of water used with changes in the surrounding plant community is also unknown (O’Keefe and Nippert 2017). Finally, previous work has shown that biodiversity can increase evapotranspiration rates, which provides support for hydrological complementarity (Verheyen et al. 2008, Kunert et al. 2012, Milcu et al. 2016). However, enhanced evapotranspiration at the community-scale likely results from greater community biomass and does not easily facilitate the partitioning of biotic from abiotic water losses from the ecosystem (De Boeck et al. 2006); thus, there is need to mechanistically understand how leaf gas exchange rates respond to varying levels of community diversity (Forrester 2015).

Here, we used the natural abundance of stable isotopes in plant and soil water to evaluate patterns of water uptake in a grassland community across an experimental diversity gradient. The stable isotopic signature of water varies predictably through a soil profile, with more enriched values (greater $\delta^{18}O$ and δD) at shallow depths and more depleted values (lower $\delta^{18}O$ and δD) at greater depths (Ehleringer and Dawson 1992). Additionally, the isotopic value of soil water is retained in non-photosynthetic plant tissue (White et al. 1985); thus, $\delta^{18}O$ and δD measured in soil and plant tissue can be used to identify the source of water utilized by plants. Plant $\delta^{18}O$ and δD can also be used to estimate ‘isotope niche space’, which is analogous to the hydrological niche area occupied by individual species and groups of species within communities.

Using this approach, we addressed the following questions: 1) does the depth-specific provenance of water uptake differ among species and/or do interspecific differences in water source manifest with increasing community diversity? 2) Does the isotopic niche space occupied by plants change with increasing diversity? 3) Is plasticity in water uptake depth across a diversity gradient associated with functional plant responses? (e.g. photosynthetic and hydraulic physiology, plant productivity).

We hypothesized that 1) hydrological niche partitioning will exist among coexisting plant species and will result from plastic shifts in depth of water uptake across a diversity gradient, 2) the isotopic niche space occupied by individual species will decrease with increasing diversity while the total isotopic niche space occupied by multiple species will increase with increasing diversity and 3) plastic shifts in water uptake across the diversity gradient will be associated with greater leaf transpiration, improved leaf water status and greater aboveground productivity for plants grown in mixtures compared to plants grown in monocultures.

Material and methods

Site description

Field work was conducted in 2017 at the Cedar Creek Ecosystem Science Reserve, a Long Term Ecological Research (LTER) site located in Bethel, MN, USA (45°35′N, 93°10′W). Cedar Creek is characterized by a variety of habitats ranging from upland oak savannas, prairies and hardwood forests, to lowland bogs, marshes and sedge meadows. Long-term mean annual precipitation (1963–2016) at Cedar Creek is 781 mm, with approximately 71% occurring during the growing season (April–September). January and July are the coldest and warmest months of the year, with average daily maximum air temperatures of −5.5°C and 28.4°C, respectively. Average daily minimum air temperatures in January and July are −16.57°C and 15.3°C, respectively. In 2017, annual precipitation was 759.7 mm, and average daily maximum and minimum July temperatures were 27.5°C and 14.6°C.

Sampling description

We sampled from the ongoing 'Big Bio' biodiversity experiment (E120), which was initiated in 1994 and consists of 168 9×9 m plots constructed on a glacial outwash sand plain (Nymore series sand, 94.4% sand, 2.5% clay; Tilman et al. 2001). This experiment manipulates the number of species grown together in plots (1, 2, 4, 8 or 16 species), and includes assemblages of C_4 grasses, C_3 grasses, C_3 forbs, legumes and woody plants (see Tilman et al. 2001 for a complete description of the experimental design).

For this study, we sampled plots containing 1, 2, 4 and 16 species. We sampled five perennial grassland species, including two C_4 grasses (*Sorghastrum nutans* and *Schizachyrium scoparium*), two non-legume C_3 forbs (*Asclepias tuberosa* and *Liatris aspera*), and one legume (*Lespedeza capitata,* which is also C_3). These species represent a range of herbaceous functional groups commonly present in grasslands and were all present in each level of the diversity treatment included in this study. Additionally, these species are all capable of rooting deeply (>2 m deep) (Weaver 1968), although deep C_4 grass roots have limited functional significance (Nippert et al. 2012).

We sampled once per month during June (DOY 151–152), July (DOY 187–189) and August (DOY 219–220) 2017. During each sampling campaign, we collected plant tissue for isotope analyses from one randomly selected individual per species in three–four plots from each diversity treatment. We also measured leaf physiological traits on one randomly selected individual per species in three–four plots from 'low' (two species) and 'high' (16 species) diversity plots. Finally, we measured soil moisture and sampled soil cores from three randomly selected plots in each diversity treatment. All species were measured during each sampling campaign except *L. aspera*, which was only measured in July and August 2017.

Microclimate data

Daily minimum and maximum air temperature (°C) and daily precipitation (mm) were recorded by a nearby weather station. Precipitation was measured with a tip bucket rain gauge. Air temperature was measured using a temperature and RH probe. All data were logged by a datalogger with a multiplexor. Additionally, volumetric soil moisture at 0–10 cm deep was measured within the soil sampling plots with a soil moisture probe. Five subsamples were measured randomly within each plot and were then averaged to obtain a single soil moisture measurement per plot.

Plant water source

The natural abundance of stable water isotopes ($\delta^{18}O$ and δD) in plant tissue and soil was used to assess depth of root water uptake by plants. Approximately 20–30 g of non-photosynthetic tissue were collected from one stem per species at each sampling plot excluding the C_4 grasses, which required 5–10 tillers to produce enough water for analyses. Samples were stored in airtight exetainer vials on ice until transferred to a 1–2°C refrigerator. One 35 cm deep soil core was collected in each soil sampling plot with a 1-inch diameter hand corer and the 0–5 cm and 30–35 cm soil sections were stored in separate exetainer vials to be used as sources in an isotope mixing model. Additionally, we collected winter precipitation (December–March) to use as a proxy for a deep water (>1 m deep) source in the mixing model. Winter precipitation was used as a deep water proxy because we were not able to collect soil at these depths and because deep water is recharged by winter precipitation and typically has a less variable isotopic signature through time (Marshall et al. 2007, Nippert and Knapp 2007).

Water was extracted from plant and soil samples using cryogenic vacuum distillation (Ehleringer and Osmond 1989, Nippert and Knapp 2007) and analyzed for stable hydrogen (δD) and oxygen ($\delta^{18}O$) signatures using an isotopic water analyzer. The lack of organic contamination of plant water samples was verified with ChemCorrect software. The δD and $\delta^{18}O$ ratios were calculated as deviations from an international standard (V-SMOW) in parts per thousand (‰) using δ-notation:

$$\delta = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \right] \tag{1}$$

where R is the ratio of the rare to common isotope for the sample and standard. The long-term precision of this instrument using in-house quality-control standards is <0.3‰ for δD and <0.15‰ for $\delta^{18}O$. Mean $\delta^{18}O$ and δD values measured for all plant, soil and precipitation samples are recorded in Supplementary material Appendix 1 Table A1, A2.

Leaf physiology

Leaf physiology, including instantaneous gas exchange and water potential, was measured on clear days adjacent to days on which isotope sampling occurred. We measured net CO_2 assimilation at ambient C_a (A_{net}), stomatal conductance of water vapor (g_s) and transpiration rate (E) using an open gas exchange system. Measurements were made on the youngest, fully expanded leaf per individual between 09:00 and 13:00 h. Cuvette conditions were set to $[CO_2] = 400\ \mu mol\ CO_2\ mol^{-1}$, flow rate $= 500\ \mu mol\ s^{-1}$ and photosynthetically active radiation $= 2000\ \mu mol\ m^{-2}\ s^{-1}$ photon flux density. Relative humidity was maintained at ambient levels. Leaves were allowed to stabilize within the chamber for approximately 3–5 min and then a single measurement was logged. Leaf area was adjusted during data processing, if required, based on measurements made in the field. Instantaneous water-use efficiency (WUE_i) was then calculated by dividing A_{net} by E.

Predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential was measured using a Scholander pressure chamber . Leaves for Ψ_{pd} were collected approximately one hour before sunrise and sealed in a dark, humidified bag for 30 min before water potential measurements were made. Leaves for Ψ_{md} were collected at noon and were also sealed in a dark, humidified bag for 30 min prior to measurement. Mean leaf gas exchange and water potential data measured for all plant samples are recorded in Supplementary material Appendix 1 Fig. A1, A2.

Plant aboveground living biomass

Plant aboveground biomass is sampled annually from each plot in the 'Big Bio' biodiversity experiment. Briefly, living biomass is clipped in narrow strips, sorted to species, dried and then weighed (Tilman et al. 2001). For our analyses, we first averaged the biomass measured in July 2017 for each species × diversity treatment combination included in this study. We then calculated the net biodiversity effect (i.e. 'overyielding') for each species (ΔY) according to the additive partitioning method (Loreau and Hector 2001, Mommer et al. 2010):

$$\Delta Y_i = Y_{Oij} - Y_{Eij} \tag{2}$$

where Y_{Oij} is the observed yield of species i in mixture j and Y_{Eij} is the expected yield of species i in mixture j. The expected yield for species i in mixture j is calculated by weighting the observed yield of species i in monoculture by the initial relative abundance of species i in mixture j. According to this method, overyielding occurs when species exhibit greater than expected biomass yields in mixed-species assemblages compared to monocultures (i.e. they have larger ΔY than monocultures).

Statistics

All statistical analyses were conducted using the statistical program R ver. 3.3.3 (<www.r-project.org>). We compared volumetric soil moisture across treatment contrasts using a three-way fixed-effects analysis of variance (ANOVA) model with diversity (1, 2, 4 and 16-species), and day of year (June, July and August sampling dates) as fixed effects.

Stable isotope analysis in R (SIAR), a Bayesian isotopic mixing model, was used to estimate the proportion of multiple water sources contributing to the dual isotopic signature ($\delta^{18}O$ and δD) of plant xylem water (Parnell et al. 2010). We ran individual analyses for each diversity treatment and included all species and sampling days within each diversity treatment. We also ran a separate set of analyses where we included all species and diversity treatments within each model, but ran individual models for each sampling date. The isotopic signatures of water collected from 5-cm deep soil, 35-cm deep soil and winter precipitation events (which represent a deep water source) were used as three sources for each model. We ran each analysis for 500 000 iterations and discarded the first 50 000 burn-in iterations. Each SIAR analysis produced a posterior distribution estimating the proportion of source water contribution for each species within that respective diversity treatment. These analyses also produced 90% Bayesian credible intervals (CI), which indicate the 90% probability that the contribution of a particular water source is within a range of values given the data (i.e. the precision of the posterior estimate) and cannot be interpreted as a test of statistical significance (Morey et al. 2016). As such, we compared posterior distributions by finding the difference between two distributions of interest. We then calculated the median of this new distribution, the proportion of the compared distributions that were different (where 0 = no difference between diversity comparisons and 1 = the diversity comparison was completely different), and the 90% credible interval (CI) of the difference between the pairwise comparisons. 90% credible intervals that did not overlap zero suggested substantial differences exist among that pairwise comparison. We also calculated the binary logarithm of the directional difference in water sources used by species growing in diverse plots (2, 4 and 16-species) relative to monocultures. Using this method, −1 = a two-fold decrease and +1 = a two-fold increase in a particular water source used by species growing in diverse plots relative to monocultures.

We used the stable isotope Bayesian ellipses in R (SIBER) package to assess whether the isotopic niche space occupied by species and groups of species changes with diversity. SIBER uses a Bayesian framework to calculate various niche metrics from bivariate isotope data (i.e. a two-dimensional 'isotopic niche' space calculated from $\delta^{18}O$ and δD) for individual species and communities (Jackson et al. 2011). First, we fit a Bayesian multivariate normal distribution to our data using Gibbs sampling in the rjags package (Plummer et al. 2018). Each model was run for 20 000 iterations and the first 1000 values were discarded. We then estimated the Bayesian standard ellipse area (SEA_B) for each species, isotopic niche space overlap between species pairs, and the Layman's convex hull area for each diversity treatment. The SEA_B produces unbiased estimates of isotopic niche area for small sample sizes and was calculated for each species growing in each diversity treatment. Isotopic niche overlap estimates the percent overlap in isotopic niche space exhibited between two different species and was calculated for each possible species pairing within each diversity treatment. The convex hull area represents the total isotopic niche area for a group of species and was calculated for each diversity treatment. We made pairwise comparisons between the posterior distributions of these estimates as described above.

To assess whether plasticity in water uptake is associated with plant functioning, we calculated the absolute difference in species-averaged water sources (5-cm, 35-cm and deep water) used between plants growing in monocultures and in higher-diversity (2- and 16-species) communities. We then assessed the relationships between leaf physiology data (A_{net}, g_s, E, Ψ_{pd}, Ψ_{md} and WUE_i) and the shift in sources for 2- and 16-species plots, as well as the relationship between biomass ΔY and the shift in sources for 2- and 16-species plots, using linear regression models. Separate models were run for each variable, with the shift in source as the predictor variable. For all analyses, we assessed homogeneity of variances by examining residuals versus fitted plots and checked for normality using normal qq-plots (Faraway 2005).

Data deposition

Data are available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.bb61h6h> (O'Keefe et al. 2019).

Results

Microclimate

Volumetric soil moisture measured at 10-cm depth did not differ across diversity treatments ($p = 0.82$) but increased across the growing season ($p < 0.01$; Supplementary material Appendix 1 Table A3). Soil moisture was driest in June (2.27 ± 0.15% SEM) and was greatest in August (6.69 ± 0.32% SEM) (Supplementary material Appendix 1 Fig. A3a). During the measurement period, mean daily maximum air temperatures were 25.64 ± 0.70, 27.46 ± 0.52 and 23.57 ± 0.51°C in June, July and August, respectively (Supplementary material Appendix 1 Fig. A3b). Maximum air temperatures for June, July and August were 0.8, 3.3 and 12.4% lower than the long-term averages (1963–2016) recorded for these months. Mean daily minimum air temperatures were 12.19 ± 0.74, 14.64 ± 0.45 and 12.37 ± 0.43°C in June, July and August, respectively (Supplementary material Appendix 1 Fig. A3b). Minimum air temperatures for June, July and August were 2.5, 4.5 and 11.5% lower than long-term monthly averages. Precipitation was lowest in July, with monthly precipitation totals of 101.6, 43.69 and 126.49 mm in June, July and August, respectively (Supplementary material Appendix 1 Fig. A3b). Precipitation was 4.4% lower in June, 58.9% lower in July and 17.6% greater in August compared to the long-term monthly precipitation totals recorded for these months.

1) Does the depth-specific provenance of water uptake differ among species and/or do interspecific differences in water source manifest with increasing community diversity?

We evaluated whether the depth of water uptake varies among common grassland species when each are grown in monoculture, or if interspecific differences in water uptake develop as communities become more diverse. First, we used a Bayesian isotope mixing model to estimate the proportional contribution of different sources to the isotopic values measured in plant xylem water and found that inherent species differences do exist among species when grown in monoculture (Fig. 1a, e, i, Supplementary material Appendix 1 Table A4). Specifically, the C_4 grasses (*S. scoparium* and *S. nutans*) used water from 5 cm-deep soil more and water from 35 cm-deep soil less compared to the C_3 forbs (*A. tuberosa* and *L. aspera*) and legume (*L. capitata*) (Fig. 1a, e). All species primarily utilized water from either 5-cm or 35-cm deep soil and did not utilize deeper soil water (Fig. 1i). Analyses with individual models for each sampling date are provided in the Supporting Information (Supplementary material Appendix 1 Table A5, Fig. A4–A7).

Next, we calculated the directional difference in source water-use between plants growing in monocultures and more diverse plots and found that most species shifted water source as community diversity increased (Fig. 2, Supplementary material Appendix 1 Table A6, A7). Generally, plants used proportionally less water from 5 cm-deep soil (>two-fold less) and more water from 35-cm deep soil in more diverse plots than in monocultures, and the greatest shifts occurred in 4- and 16-species plots (Fig. 2a–b). Plants also used proportionally less deep water in more diverse communities compared to monocultures, with the greatest reductions occurring in *A. tuberosa* (Supplementary material Appendix 1 Table A6). Although the magnitude of plasticity varied among species, all species generally shifted water uptake in the same direction across the diversity gradient. This resulted in similar interspecific differences in water uptake patterns across communities (Fig. 1). In other words, grasses always used shallower water than forbs, regardless of shifting water source across the diversity gradient. However, the magnitude of interspecific differences varied among communities (e.g. although grasses always used proportionally less water from 35-cm depth than forbs, this difference was greater in four-species communities than in two-species communities). *S. nutans*, in particular, exhibited the greatest shifts in water source and this trend was greatest in the four-species communities (Fig. 2). Our models that considered each sampling period separately produced similar results, but also revealed that shifts in water uptake from 5-cm water to 35-cm water became more pronounced towards the end of the growing season (Supplementary material Appendix 1 Table A8, Fig. A8).

2) Does the isotopic niche space occupied by plants change with increasing diversity?

We also used a Bayesian modelling approach to assess whether the isotopic niche space occupied by species and groups of

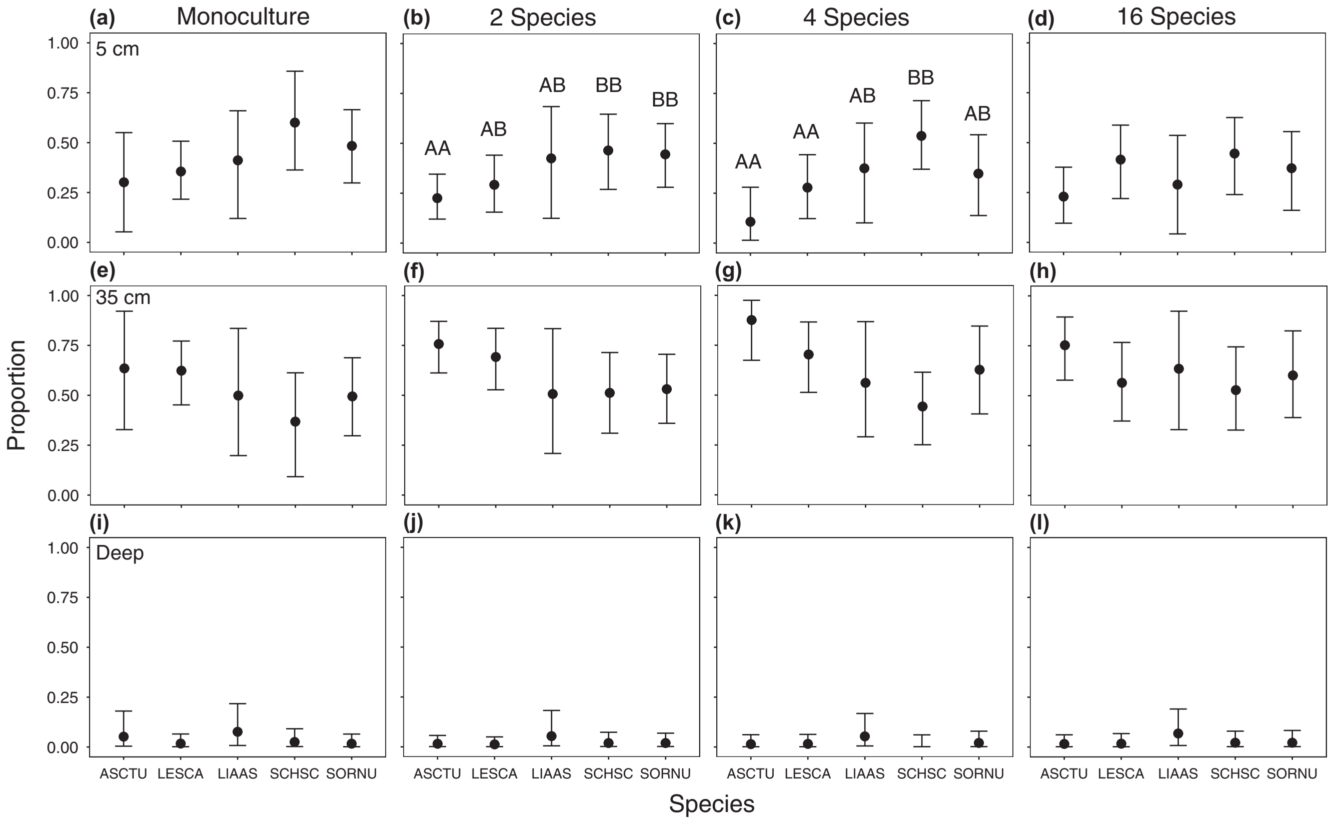

Figure 1. Median (±90% credible interval) posterior density estimates for proportional contribution of 5 cm (a–d), 35 cm (e–h) and deep (i–l) source water to plant xylem water. Shown are estimates for *A. tuberosa* (ASCTU), *L. capitata* (LESCA), *L. aspera* (LIAAS), *S. scoparium* (SCHSC) and *S. nutans* (SORNU), growing in 1 (a, e, i), 2 (b, f, j), 4 (c, g, k) and 16 (d, h, l)-species plots. Letters indicate that the 90% credible intervals of the difference between pairwise comparisons did not overlap zero, which suggests substantial differences exist among those species. See Supplementary material Appendix 1 Table A4 for all pairwise comparison statistics.

species changes with diversity. In general, shifts in isotopic niche area across the diversity gradient were subtle and varied by species. The Bayesian standard ellipse area (SEA_B), which describes the isotopic niche area of individual species, was greatest in monoculture plots for *A. tuberosa*, *L. aspera* and *S. scoparium* (Supplementary material Appendix 1 Table A9, Fig. A9a, c, d). For instance, the mean SEA_B estimated for *S. scoparium* grown in monocultures was 66.41‰^{2}, but

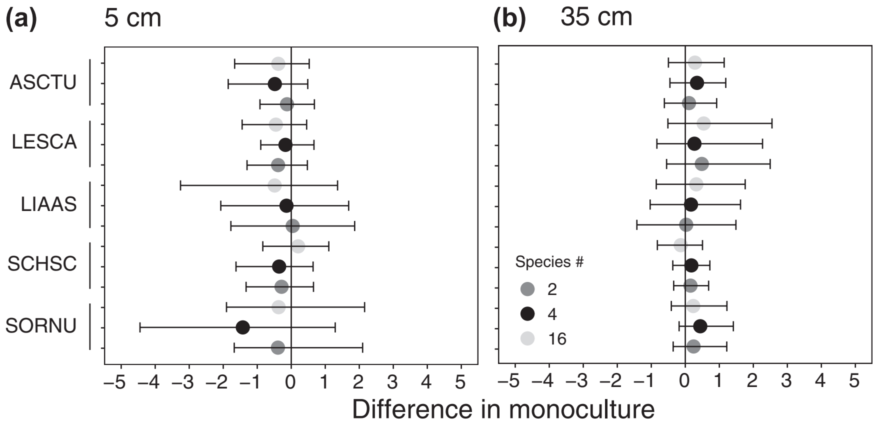

Figure 2. Difference in 5 cm (a) and 35 cm (b) water sources used by *A. tuberosa* (ASCTU), *L. capitata* (LESCA), *L. aspera* (LIAAS), *S. scoparium* (SCHSC) and *S. nutans* (SORNU) growing in diverse plots (2, 4 and 16-species) relative to monocultures. Shown is the median binary logarithm of the relative difference (±90% credible interval) between a particular water source used in a diverse plot compared to a monoculture plot, where −1 = a two-fold decrease and +1 = a two-fold increase in a water source used relative to a monoculture. See Supplementary material Appendix 1 Table A7 for pairwise comparison statistics.

only ranged between 32.54 and 44.70‰^{2} in more diverse plots (Supplementary material Appendix 1 Fig. A9d), which indicates a narrowing of niche space as diversity increased. Conversely, SEA_B was greatest in *L. capitata* grown in 16-species plots and did not vary in *S. nutans* across the diversity gradient (Supplementary material Appendix 1 Fig. A9b–e). The overall magnitude of SEA_B also varied across species, with *L. aspera* generally having greater niche area than other species (Supplementary material Appendix 1 Fig. A9c).

The overlap in niche area occupied by different species pairs varied little across diversity treatments. Of the shifts that did occur with changes in diversity, niche area overlap was somewhat greater in more diverse communities compared to monocultures (Supplementary material Appendix 1 Table A10, Fig. A10). For example, the amount of isotopic niche space occupied by both *A. tuberosa* and *S. scoparium* increased from 0.17% in monocultures to 0.32% in 16-species plots (Supplementary material Appendix 1 Fig. A10i). The overlap between *L. capitata* and *S. scoparium* also increased from 0.25% in monocultures to 0.51% in 16-species plots (Supplementary material Appendix 1 Fig. A10e). Among all the species pairs, the greatest overlap in isotopic niche area occurred between the two grasses (Supplementary material Appendix 1 Fig. A10a), and the least overlap occurred between *A. tuberosa* and *S. scoparium* (Supplementary material Appendix 1 Fig. A10i).

We also estimated the Layman's convex hull area for the five species in each diversity treatment, which indicates the total isotopic niche space occupied by these species combined. Convex hull area was similar across all diversity treatments, ranging only from 3.43‰^{2} in four-species plots to 7.78‰^{2} in two-species plots (Supplementary material Appendix 1 Table A11, Fig. A11).

3) Is plasticity in water uptake depth across a diversity gradient associated with functional plant responses?

We assessed whether plasticity in water uptake across a diversity gradient is associated with plant functioning by comparing absolute shifts in water sources (5-cm, 35-cm and deep water) with leaf-level physiology and biomass overyielding. Leaf transpiration, stomatal conductance and midday leaf water potential declined significantly with greater absolute shifts in 5-cm water-use (Fig. 3b, c, e, Supplementary material Appendix 1 Table A12). Conversely, instantaneous water-use efficiency and biomass overyielding increased significantly with greater shifts in 5-cm water-use (Fig. 3f, 4, Supplementary material Appendix 1 Table A12). There was no significant relationship between photosynthesis and shifts in 5-cm water-use, or between predawn leaf water potential and shifts in 5-cm water-use (Fig. 3a, d). Relationships between plant traits and shifts in other sources (35-cm and deep water) were also generally weak (Supplementary material Appendix 1 Table A12).

Discussion

Although biodiversity enhances numerous ecosystem processes, the underlying mechanisms driving this relationship are not yet fully understood and are typically not investigated (Hooper et al. 2005). Here, we used a stable isotope approach to assess whether niche partitioning of water occurs across an experimentally manipulated diversity gradient and if this mechanism has the potential to enhance plant functioning in diverse grassland communities. We found that inherent species differences in water uptake exist in monocultures, and that species generally shift reliance from shallow soil layers to intermediate-depth (35-cm deep) soil water with increasing community diversity. Greater plasticity in water uptake across the diversity gradient is also associated with declines in certain plant physiological traits (e.g. leaf E, g_s and Ψ_{md}), as well as greater than expected biomass production and increased leaf WUE_i. Overall, these results suggest that niche partitioning for water resources, as well as plasticity in water-use, may act as mechanisms contributing to the positive biodiversity–productivity relationship previously shown in this experimental grassland.

1) Does the depth-specific provenance of water uptake differ among species and/or do interspecific differences in water source manifest with increasing community diversity?

Vertical partitioning of soil resources has long been proposed as a mechanism for species coexistence in natural ecosystems (Walter 1939, Walker and Noy-Meir 1982, Silvertown 2004, Nippert and Holdo 2014). According to this hypothesis, roots that occupy different spatial niches have access to different soil resources, limiting competition between co-occurring plants and promoting the stable coexistence of multiple species within a plant community (MacArthur and Levins 1967, Silvertown et al. 1999). Numerous studies have supported this idea by showing differences in rooting depth and depth of water uptake among co-existing species in both herbaceous (Asbjornsen et al. 2008, Herben et al. 2017) and woody ecosystems (Schume et al. 2003, Schwendenmann et al. 2015). For instance, previous work in grasslands has shown that, similar to our results, deep-rooted forbs and shrubs tend to use deeper water sources than grasses, which rely almost exclusively on shallow soil water (Nippert and Knapp 2007, Priyadarshini et al. 2015).

Importantly, these findings have been made primarily in natural ecosystems that typically contain many coexisting, and potentially interacting, plant species. Studies that show vertical niche partitioning for resources in natural ecosystems therefore cannot disentangle the two possibilities that interspecific differences in resource use would still occur in the absence of interspecific competition, or that niche differentiation develops in response to competition by neighboring plants for the same resources. Given that plant roots can alter growth patterns in response to neighbors (Wardle and Peltzer 2003, Bolte and Villanueva 2006, Mommer et al. 2010), plants may concomitantly shift

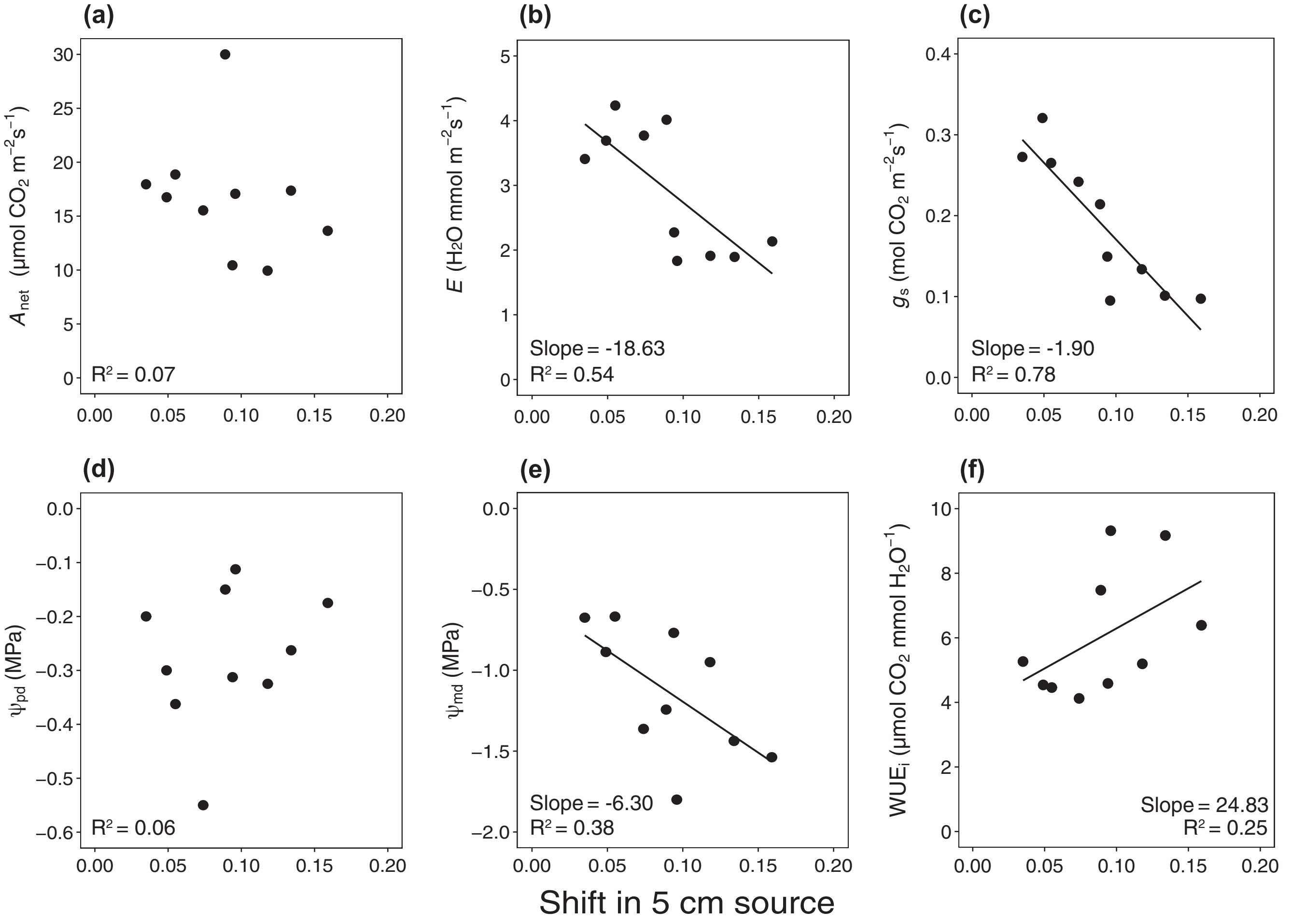

Figure 3. Linear regression relationships between plant traits and the shift in proportion of 5 cm water source used by plants growing in diverse plots relative to monocultures. Shown are R^2 values and slopes of significant relationships for photosynthesis, A_{net} (a), transpiration, E (b), stomatal conductance, g_s (c), predawn leaf water potential, Ψ_{pd} (d), midday leaf water potential, Ψ_{md} (e) and instantaneous water-use efficiency, WUE$_i$ (f). See Supplementary material Appendix 1 Table A12 for all regression statistics.

depth of water uptake when grown in mixed assemblages. Here, we provide novel evidence that patterns of root water uptake are innately different among species when grown in monocultures (Fig. 1a, e, i), and also that plant species reduce reliance on shallower soil water sources when their surrounding community becomes more diverse (Fig. 2). However, all species showed proportional reductions in use of water from the shallowest depths and proportionally more use from intermediate-depths in mixtures, leading to similar interspecific differences in water uptake across communities (Fig. 1). Thus, niche partitioning of water within this grassland is a function of inherent species differences in water-use, independent of plastic responses to the surrounding community.

Our results are somewhat similar to those of previous studies that have measured plant resource-use in other experimental grasslands. Lipowsky et al. (2015), for instance, showed that plant traits associated with light and nutrient acquisition were inherently different among grassland forbs and also exhibited plastic responses to increasing species richness at the Jena Experiment in Germany. Herben et al. (2017) used molecular techniques to demonstrate distinct differences in rooting depth among coexisting species within an experimental grassland located in the Czech Republic; they also found that differences in rooting depth were inherently different among species and did not result from interspecific competition between neighboring plants. Contrary to our results, Bachmann et al. (2015) found no differences in depth of water uptake across different plant functional groups or across an experimental grassland diversity gradient. However, this study did not assess interspecific differences in water uptake, which may exist despite the lack of variation measured across functional groups. We also measured lower soil water content at the Cedar Creek Biodiversity Experiment (Supplementary material Appendix 1 Fig. A3a) than Bachmann et al. (2015) did at the Jena Experiment. Differences in soil water content may explain the incongruent results found between Bachmann et al. (2015) and our study because deep-rooted plants often shift reliance to deeper soil water during dry periods when shallow soil water is limiting

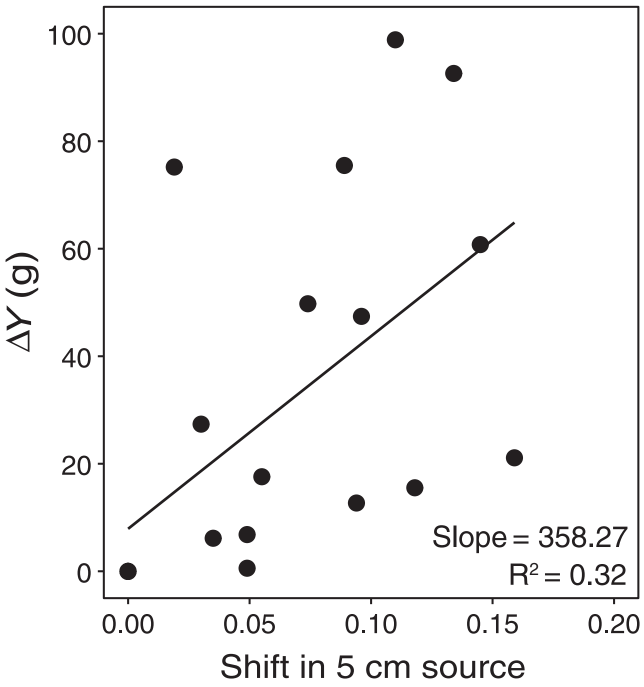

Figure 4. Linear regression relationship between the biodiversity effect on biomass yield, Δ*Y*, and the shift in proportion of 5 cm water source used by plants growing in diverse plots relative to monocultures. Shown are R^2 and slope values. See Supplementary material Appendix 1 Table A12 for all regression statistics.

and transpirational demands are high (Nippert and Knapp 2007, Guderle et al. 2017). As such, the drier shallow soils observed in our study may have influenced deep-rooted forbs to utilize deeper soil water, enhancing niche partitioning among species. Diversity-induced shifts in water source may also only develop under dry conditions, as was illustrated by Guderle et al. (2017); this study reported deeper soil water-use by plants growing in more diverse plant communities, but only during periods of high leaf-to-air vapor pressure deficit.

2) Does the isotopic niche space occupied by plants change with increasing diversity?

Plastic responses to diversity can potentially alter the niche space occupied by individual species, as well as the degree of niche overlap occurring between species (Lipowsky et al. 2015, Weisser et al. 2017). Although we hypothesized that plants growing in more diverse communities would occupy smaller isotopic niches than when grown in monocultures, we found only subtle, species-specific changes in isotopic niche space across the diversity gradient (Supplementary material Appendix 1 Fig. A9). Likewise, we found only small increases in niche overlap among species growing in diverse communities compared to monocultures (Supplementary material Appendix 1 Fig. A10). These results are consistent with our finding that all species experienced similar shifts in water uptake, and consequently exhibited similar interspecific differences in water-use across the diversity gradient (Fig. 1, 2). If water uptake responses to diversity had been more variable among species, changes in isotopic niche area and/or niche overlap may have been more evident in our study. Alternatively, subtle shifts in water uptake with changes in diversity may be more easily detected with isotope labeling techniques.

Our finding that multiple species shifted reliance to intermediate-depth water sources with increasing diversity is consistent with previous findings that show vertical root biomass increases with species diversity (von Felten et al. 2009, Mommer et al. 2015, Guderle et al. 2017). Community-level rooting depth is indeed greater in multi-species assemblages compared to monocultures at this site, which may be driven by the presence of deep-rooted legumes that increase nitrogen availability in deeper soils (Mueller et al. 2013). Nitrogen-driven increases in rooting depth may consequently facilitate the greater reliance on intermediate-depth water shown here (Fig. 2). Considering the greater spatial distribution of roots in more diverse mixtures, it is interesting that we found no differences in multiple species-level niche area across the diversity gradient (Supplementary material Appendix 1 Fig. A11). However, our analysis only included 5 of 16 total species within the Cedar Creek Biodiversity Experiment, which may not capture the entire isotopic niche space of the most diverse communities. The species we did measure also all exploited proportionally more intermediate-depth soil water in mixtures, which would restrict the total niche area considered for multiple species and supports the idea that greater rooting depth does not improve community functioning when most species utilize water from one particular depth (Oram et al. 2018). Similarly, others have found that depth of water uptake does not always correspond with rooting depth and is more often driven by resource availability (Kulmatiski et al. 2017) or root hydraulic properties (Nippert et al. 2012).

3) Is plasticity in water uptake depth across a diversity gradient associated with functional plant responses?

Enhanced ecosystem evapotranspiration is often reported in mixed-species assemblages and is used as evidence to suggest that hydrological niche complementarity leads to more complete soil water extraction in diverse communities compared to monocultures (Schume et al. 2003, Verheyen et al. 2008). These patterns are typically attributed to greater leaf area in more diverse plant communities (De Boeck et al. 2006), although some studies have used leaf $\delta^{13}C$ data to suggest that leaf g_s also increases in mixed-species communities (Caldeira et al. 2001, Lipowsky et al. 2015, Weisser et al. 2017). In contrast, we show that leaf E, g_s and Ψ_{md} were all lower in plants that experienced greater shifts in water source-use relative to monocultures (Fig. 3b, c, e). While we can only speculate about the role of intermediate-depth soil water on plant functioning in this experiment because we do not have soil moisture measurements at depths greater than 10-cm, greater proportional reliance on soil water at 35-cm by more diverse mixtures may quickly deplete intermediate-depth soil moisture and consequently lower leaf g_s and E for species that primarily access water at this depth.

The concomitant decline in leaf Ψ_{md} and leaf g_s could also be explained by a decrease in whole-plant conductance, which may result from a longer path length for water flow associated with deeper roots (Ryan and Yoder 1997).

Although E and g_s were lower in species with increased plasticity in root water uptake, leaf A_{net} did not vary significantly with plasticity in water-use and WUE_i was consequently greater in these species (Fig. 3a, f). Maintenance of leaf A_{net} despite concomitant reductions in g_s could be explained by the greater availability of soil nitrogen found in diverse assemblages at this site (Zak et al. 2003, Fargione et al. 2007, Dybzinski et al. 2008). Soil nitrogen is generally greater in diverse communities due to the presence of nitrogen-fixing legumes and may enhance plant growth in biodiversity experiments (Tilman et al. 1996, Lambers et al. 2004). Thus, the greater concentration of nitrogen in a system that is primarily nitrogen-limited (Tilman 1984) may increase WUE_i in plants that are able to exhibit plastic shifts in rooting depth and access water and nitrogen at greater depths.

Despite these contrasting leaf physiological responses, we found a significant positive relationship between biomass overyielding and plasticity in sources of water used (Fig. 4). Previous biodiversity–ecosystem functioning studies have demonstrated that biomass overyielding commonly occurs in diverse communities, and this pattern is often attributed to niche complementarity without directly measuring resource-use (Loreau et al. 2001, Lambers et al. 2004, Fargione and Tilman 2005, Cardinale et al. 2007). However, overyielding is not necessarily direct evidence for niche partitioning because other positive factors, such as facilitation, could also enhance overyielding in mixtures (Loreau and Hector 2001, Wright et al. 2017). Here, we show that the ability to vary water sources from monocultures to mixtures (Fig. 2) may contribute to positive biodiversity–productivity patterns at this site. As discussed above, plasticity in root water uptake may result from plasticity in root growth (Mueller et al. 2013), which could allow plants to extract water and nitrogen where it is available (Kulmatiski et al. 2017). This, in turn, may increase WUE_i and result in the overyielding observed here (Fig. 3, 4).

Ecological considerations and conclusions

Mounting evidence suggests that biodiversity not only increases plant productivity, but also stabilizes productivity over time (Lehman and Tilman 2000, Isbell et al. 2009) and through climatic disturbances (Hallett et al. 2017, Kreyling et al. 2017, Wagg et al. 2017). Recently, Anderegg et al. (2018) reported that hydraulic trait diversity also enhances forest ecosystem stability in response to water availability. Although Anderegg et al. (2018) only considered hydraulic traits related to efficiency and safety, it is reasonable to hypothesize that interspecific differences in water-use, as well as plasticity in water uptake, may also enhance ecosystem stability in a changing climate for both forests and herbaceous systems. Reliance on deeper water sources, in particular, could stabilize diverse ecosystems during drought because deep soil water availability is generally stable through time (Craine and Nippert 2013) and has the potential to buffer negative physiological responses to stressful environmental fluctuations (Nippert et al. 2013). Conversely, more complete utilization of soil water by diverse communities could enhance their susceptibility to drought (Pfisterer and Schmid 2002, Verheyen et al. 2008). It is important to note, however, that water-use dynamics and plasticity in hydraulic traits likely differ in forests compared to grasslands, and future work should investigate these dynamics in a wider range of ecosystems. Given that plant functioning is often coupled with ecosystem carbon and water fluxes (Petrie et al. 2012, Zeppel et al. 2014), understanding how biodiversity influences plant responses to the environment will be critical for forecasting grassland responses to a changing climate.

While many studies hypothesize that niche complementarity drives positive biodiversity–ecosystem functioning patterns, few have actually demonstrated direct experimental evidence of resource-use partitioning in biodiversity experiments. In contrast, we show that niche partitioning for water-use occurs in an experimental grassland and results from inherent interspecific differences among plant species. We also show that a common decline in the depth of water uptake across species is associated with greater than expected biomass production. Thus, plasticity in water-use is likely one of the mechanisms driving the positive biodiversity–productivity relationship observed in this system. Considering that plants are often limited by multiple resources (Harpole and Tilman 2007), plasticity in root water uptake does not likely fully explain biodiversity–productivity relationships in grasslands (Bachmann et al. 2015). We suggest that the presence of deep-rooted legumes in diverse mixtures increases nitrogen concentration and root growth at greater depths (Mueller et al. 2013), which consequently promotes the deeper water-use and concomitant shifts in physiology (i.e. decreased Ψ_{md} and increased WUE_i) observed here. Future research should investigate partitioning for multiple limiting resources (e.g. water and nitrogen), over longer periods of time and in response to greater climatic variability.

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Conflicts of interest – We have no conflicts of interest to declare.

Author contributions – KO and KAM conceived the ideas and designed methodology; KO collected the data, analyzed the data and wrote the manuscript; KO, JBN and KAM all contributed critically to the drafts and gave final approval for publication.

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Supplementary material (available online as Appendix-oik-06529 at <www.oikosjournal.org/appendix/oik-06529>). Appendix 1.

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