

Title: Does plant diversity affect the water balance of established grassland systems?

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Abstract

Evidence from experimental grasslands indicated that plant biodiversity modifies the water cycle but it is unclear if this is also true for established land-use systems. Therefore, we investigated how evapotranspiration (ETa), downward flux (DF), and upward flux (UF) in soil are related with land use and plant diversity in agriculturally managed grassland. In three Central European regions (“Biodiversity Exploratories”), we studied 29 grassland plots (50m x 50m; 9-11 plots per region) covering the land-use classes pasture, mown pasture, and meadow in at least triplicate per region. From 2010 to 2015, we measured soil moisture, meteorological conditions, plant species richness, cover and number of species in the functional groups of grasses, herbs, and legumes, aboveground biomass and root biomass on each plot. Annual ETa , DF , and UF were calculated for two soil layers with a soil water balance model and statistically analyzed for land-use and biodiversity effects with analysis of variance. Water fluxes were not significantly affected by land-use class. UF did not vary between plots with different species richness and plant functional group composition. DF from topsoil increased with increasing number of grass species. ETa from topsoil decreased

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with increasing species richness and with the number of herb or legume species, while ET_a from subsoil increased. Our results demonstrate that plant diversity influences the soil depth partitioning of water use, but the complex drivers of this relationship in agriculturally managed grassland still need to be disentangled.

Keywords: Biodiversity Exploratories, biodiversity, land use, evapotranspiration, upward flux, downward flux, soil water flux

Accepted Article

1 Introduction

Biodiversity plays an important role for the supply of ecosystem services (Cardinale et al., 2012; Isbell et al., 2011; Soliveres et al., 2016). Drinking water supply via groundwater recharge is one of the ecosystem services, which is highly demanded by the public.

Additionally, the water cycle is an important control of nutrient cycles and plant productivity because nutrients are transported with water and plants require water and nutrients for biomass production. As biodiversity is globally declining because of several reasons including land-use intensification (Butchart et al., 2010; Foley et al., 2005; Sala et al., 2000), it is important to understand the biodiversity-water cycle relationship. While the influence of land use on the water cycle has been intensively studied (e.g. Fatichi, Zeeman, Fuhrer, & Burlando, 2014; Leitinger, Tasser, Newesely, Obojes, & Tappeiner, 2010), the influence of land use and associated biodiversity on the water cycle in established grassland systems is unclear.

Grassland management can influence the water balance. Grazing and low grass cuts by mowing can lead to reduced evapotranspiration and increased downward water fluxes (Fatichi et al., 2014; Inauen, Körner, & Hiltbrunner, 2013; Li et al., 2015). The mechanism behind this relationship is probably that reduced biomass decreases interception loss and transpiration, but also leads to increased soil evaporation (Fatichi et al., 2014). Furthermore, animal treading usually causes soil compaction (Fatichi et al., 2014), which decreases the infiltration capacity (i.e. maximum infiltration rate under given conditions) of the soil (Leitinger et al., 2010).

From manipulative biodiversity experiments, in which mainly plant species richness is experimentally varied and other site characteristics and management are kept constant, we know that increasing plant species richness increases community productivity (Hector et al., 1999; Marquard et al., 2009; Spehn et al., 2005; Tilman et al., 2001). As transpiration increases with productivity, this leads to higher evapotranspiration in diverse mixtures (De Boeck et al., 2006; Verheyen et al., 2008). However, plant cover also increases with species richness (Spehn, Joshi, Schmid, Diemer, & Körner, 2000; Tilman, Wedin, & Knops, 1996) and might compensate the increase in transpiration by stronger shading of the soil, resulting in reduced soil evaporation and subsequently no change in evapotranspiration with plant diversity (Leimer et al., 2014; Spehn, Joshi, Schmid, Alpehi, & Körner, 2000). Increasing root biomass with increasing plant species richness (Ravenek et al., 2014) allows for more exhaustive water use and therefore decreases downward water fluxes (i.e. loss of water to the deeper soil or groundwater) and increase upward water flux (i.e. water input from deeper soil layers or groundwater via capillary rise). The soil organic carbon stock, which is also important for the soil water cycle because of the well known relationship between organic carbon concentrations and the soil water holding capacity, increases with increasing plant species richness (Lange et al., 2015). On the other hand, soil bulk density decreases and infiltration capacity increases with increasing species richness (C. Fischer et al., 2015), possibly leading to less soil evaporation and increased downward water fluxes from topsoil to subsoil in species-rich plant mixtures.

In contrast to experiments, in which species richness is artificially varied, species richness in agriculturally managed grassland is driven by the kind of land use. Fertilizer application, grazing, and mowing strongly affect plant species richness and plant functional group composition in established and agriculturally managed grassland systems. Fertilizer application usually decreases species richness while it increases biomass (Crawley et al., 2005; Gough, Osenberg, Gross, & Collins, 2000; Socher et al., 2012). Fertilizer application can also increase the cover of grasses, proportion of grass species and reduce the proportion

of herb species (Socher et al., 2012, 2013). Grazing intensity, livestock types, and mowing frequency can further influence plant species richness and functional composition. However, these relationships vary with the studied region (Collins, Knapp, Briggs, Blair, & Steinauer, 1998; Klaus et al., 2013; Socher et al., 2013; Zechmeister, Schmitzberger, Steurer, Peterseil, & Wrבka, 2003). Agricultural management varies across land-use classes, like pasture, mown pasture, and meadow and subsequently species richness, cover and proportion of plant functional groups vary across land-use classes (Socher et al., 2012, 2013).

Different biological plant traits, which can be related to plant functional groups, also modify the water uptake of plants from soil. Such traits include the leaf morphology, which influences the degree of shading and thereby modifies soil evaporation, but also the root distribution and rooting depth (Jarvis, 1989; Leimer et al., 2014; Prasad, 1988). For example, plant mixtures containing grasses are reported to decrease downward water flux and increase evapotranspiration from topsoil compared to mixtures without grasses because of the extensive, shallow rooting system and the leaf morphology of grasses, which causes less shade. In contrast, mixtures containing legumes increase downward water flux and decrease evapotranspiration from topsoil because of deeper roots, which facilitate water use from deeper soil layers, and higher biomass production caused by increased nitrogen availability, which reduces soil evaporation from topsoil (Leimer et al., 2014).

From manipulative biodiversity experiments, it has been reported that species richness and the presence/absence of plant functional groups affect soil water contents and soil water fluxes in topsoil and subsoil in opposing directions (Caldeira, Ryel, Lawton, & Pereira, 2001; Leimer et al., 2014). This is likely caused by variations of plant community properties with soil depth (e.g. rooting depth or presence/absence of taproots) which are related with species richness or plant functional traits (Caldeira et al., 2001; Leimer et al., 2014). Therefore, it is important to differentiate between topsoil and subsoil when studying the plant diversity-water cycling relationship.

To the best of our knowledge, only Obojes et al. (2015) investigated vegetation effects on evapotranspiration from established real-world systems (i.e. mountain grasslands in the Alps). Increasing abundance of tall grasses increased evapotranspiration. Obojes et al. (2015) further reported indications for vegetation effects on the variability of downward water fluxes below 28 cm soil depth. However, downward water fluxes were not further evaluated and upward water flux was not determined in any study we are aware of.

The main objective of our study was to investigate, if soil water fluxes (evapotranspiration, downward flux, and upward flux) are related with plant diversity in established and agriculturally managed Central European grassland systems. (1) We hypothesize that annual water fluxes (ET_a , DF , UF) are affected by land use, with reduced evapotranspiration and increased downward water flux in the grazed plots of lower land-use intensity compared with the high intensity meadow management. According to previous findings in manipulative biodiversity experiments, we further hypothesize: (2) After accounting for the factors region and land use, plant species richness modifies water fluxes with contrasting effects on topsoil and subsoil. We expect that increasing species richness significantly decreases evapotranspiration from topsoil and increases evapotranspiration from subsoil. (3) Annual water fluxes vary with plant functional group composition (i.e. cover and species number of grasses, legumes, and herbs). We expect grasses to increase evapotranspiration and decrease downward water fluxes from topsoil, whereas cover and number of legumes decrease evapotranspiration from topsoil and increase downward water fluxes from topsoil and evapotranspiration from subsoil.

2 Methods

This study was conducted as part of the “Biodiversity Exploratories” project (www.biodiversity-exploratories.de) in three regions in Germany: Schorfheide-Chorin (SCH) is situated in the lowlands of northeastern Germany representing a young glacial landscape with many wetlands (elevation of studied plots: 16-73 m, annual mean temperature: 8-8.5°C, soils: Histosols, Gleysols, Cambisols). Hainich-Dün (HAI) is situated in the hilly lands of central Germany with calcareous bedrock (elevation of studied plots: 285-450 m, annual mean temperature: 6.5-8°C, soils: Stagnosols, Vertisols, Cambisols). The Schwäbische Alb (ALB), also known as Swabian Jura, is situated in the low mountain ranges of southwestern Germany with calcareous bedrock and karst phenomena (elevation of studied plots: 660-808 m, annual mean temperature: 6-7°C, soils: Leptosols and Cambisols) (M. Fischer et al., 2010). Climate, geological setting and prevalent soil types of the three regions are representative for large parts of Central Europe. We investigated 11 agriculturally managed grassland plots in SCH, 9 in HAI, and 9 in ALB (each 50 m x 50 m) comprising the land-use classes pasture, mown pasture, and meadow in at least triplicate per region (for details see M. Fischer et al., 2010). On average, the studied pastures were managed with lowest land-use intensity in the study period compared to the mown pastures with medium and the meadows with highest land-use intensity (see Fig. S1).

2.1 Field measurements

2.1.1 Climate data

On each of the 29 plots, we measured relative humidity [%] and air temperature [°C] at 2 m above ground and soil moisture [Vol.-%] in 0.10, 0.15, 0.20, and 0.30 m soil depth in hourly resolution from 2010 to 2015. The data has been quality controlled with respect to physical validity and temporal dynamics of the recorded values at a specific station and in relation between the stations.

Precipitation [mm] was determined at intervals of 14 days with 5 collectors per site placed at 1.8 m height. Collectors for rainfall consisted of 2-L polyethylene bottles with a polyethylene funnel (diameter = 0.12 m) at the top. Funnels were equipped with a polyester net (1.6 mm mesh width) and a table-tennis ball to minimize evaporation. Sampling was interrupted in winter (approximately December to February). These regular interruptions and other irregular data gaps resulted in 46 % of the 4553 necessary biweekly precipitation values to be missing.

To replace these missing values in the biweekly precipitation dataset, we used daily precipitation data provided by the DWD Climate Data Center (2017). We selected the station Münsingen-Apfelstetten (ID: 3402) for the region Schwäbische Alb, Südeichsfeld-Wendehausen (ID: 5084) for Hainich-Dün, and Friedrichswalde (ID: 1497) for Schorfheide-Chorin. Biweekly aggregated precipitation data from the respective DWD station was well correlated with plot-specific measurements (Pearson’s r was 0.60-0.81 for SCH, 0.49-0.70 for HAI, and 0.83-0.95 for ALB).

2.1.2 Plant data

Root biomass [g] was determined in 2007 in 0-0.1 m, 0.1-0.3 m, and below 0.3 m soil depth if roots were present. We calculated the depth-weighted mean root biomass per plot for the 0-0.15 m soil layer, total root biomass per plot, and subsequently the proportion of roots in the 0-0.15 m soil layer out of total root biomass. On average 87 % (ALB), 89 % (HAI), and 70 % (SCH) of the total root biomass were in the 0-0.15 m soil layer.

Vegetation was recorded annually from 2010 to 2015 on each plot. We estimated the cover of all vascular plant species on 4 m x 4 m from mid-May to mid-June simultaneously in all three regions and calculated plant diversity as the number of plant species per 16 m² (Tab. S2). Cover sums of the functional groups grasses, legumes and herbs were calculated by summing up the cover of all species in the respective groups. Although plots were regularly managed by resident farmers, temporary fences ensured that no mowing or grazing took place prior to sampling (Klaus et al., 2013). Aboveground community biomass was sampled by cutting the vegetation at a height of 0.02-0.03 m in four 0.5 m × 0.5 m subplots. Biomass was oven-dried for 48h at 80°C, weighed immediately after drying, and averaged per plot.

2.2 Data preprocessing

2.2.1 Potential evapotranspiration

Daily potential evapotranspiration (ET_p) was calculated from 2010 to 2015 per plot according to Haude (1955) and corrected for the land-use type “hay meadow” as the closest approximation of the studied land-use systems (DVWK, 1996). The equation is based on air temperature and relative humidity at 2:30 pm, which were calculated as mean of the measurements at 2:00 pm and 3:00 pm. Daily ET_p was aggregated to the same 14-day periods, for which the plot-specific precipitation measurements were available. Missing data accounted for 22 % of the biweekly ET_p data.

Data gaps in calculated ET_p were filled with Bayesian modeling per region because a complete dataset is necessary to calculate the annual water budget. We developed a model that is capable of simulating biweekly ET_p per plot, based on available ET_p data from other plots in the respective region. For model development, a Bayesian statistical approach was chosen because it allows for missing values in the explanatory variables, considers measurement uncertainty, and needs less parameterization effort than a process-oriented model. We used the program OpenBUGS (Lunn, Spiegelhalter, Thomas, & Best, 2009) to set up a Bayesian model for each region. The mean of ET_p per time step t of all grassland plots per region plus a plot-specific parameter a_p turned out to be most suitable for simulating missing ET_p values per plot p in this study (Eq. 1). A normal distribution with mean (μ_a) 0 and precision τ_a was assumed for the parameter a (Eq. 2).

$$ET_{p,t} \sim Normal(\mu_{ET_p} = mean(ET_{p,t}) + a_p, \tau \sim Gamma(0.001, 0.001)) \quad (1)$$

$$a_p \sim Normal(\mu_a = 0, \tau_a \sim Gamma(0.001, 0.001)) \quad (2)$$

Convergence of the models (one per region) occurred at approx. 2,000 updates. For each model, convergence was checked using three chains with different random starting values and the modified Gelman-Rubin statistic as implemented in OpenBUGS. We used 10,000 iterations as “burn-in”. For each model, one of the chains was run for further 10,000 iterations on which the estimates of the missing ET_p values and parameters are based. Only missing ET_p values were replaced with the mean of the estimations and available calculated values were kept as calculated from the climate data with the Haude formula. Model quality was assessed using several quality measures. The model for ET_p performed well in all three regions (see supporting information).

2.2.2 Soil water content

Soil water contents per plot were aggregated to depth-weighted means for the 0-0.15 m (ALB, HAI, SCH), 0.15-0.35 m (HAI), and 0.15-0.70 m (SCH) soil layers and converted to soil water storage [mm]. The soil layers were chosen according to soil thickness, rooting depth, and the depth in which suction cups were installed. In ALB, soils are shallow and

frequently do not reach deeper than 0.15 m (4 of 9 plots). Therefore, no investigation of subsoil was possible in ALB. Only data from Tuesday noon every second week was used, which is in agreement with the sampling dates of plot-specific precipitation and allows for the calculation of the change in soil water storage between sampling dates. Missing data accounted for 12 % of the data for 0-0.15 m (ALB, HAI, and SCH), 5 % of the data for 0.15-0.35 m (only HAI), and 20 % of the data for 0.15-0.70 m (only SCH).

Gaps in soil water storage data were filled per region with a Bayesian model based on Leimer et al. (2014). In brief, we used linear regression-type models per region with the mean of all plots in the considered region and soil depth per time step t as explanatory variable to estimate missing plot- and soil layer-specific soil water storage (S ; Eq. 3). Assumptions for parameter a were the same as in Section 2.2.1 (Eq. 4). To ensure that only reasonable values are estimated for the parameter b (i.e. the slope in the linear regression), a uniform distribution with minimum 0 and maximum 2 was chosen (Eq. 5).

$$S_{t,p} \sim Normal(\mu_S = a_p + b_p \cdot \text{mean}(S_t), \tau \sim Gamma(0.001, 0.001)) \quad (3)$$

$$a_p \sim Normal(\mu_a = 0, \tau_a = Gamma(0.001, 0.001)) \quad (4)$$

$$b_p \sim Uniform(0, 2) \quad (5)$$

The same model structure was used to simulate soil water storage per plot (p) in the 0-0.15 m (ALB, HAI, SCH), 0.15-0.35 m (HAI), and 0.15-0.70 m (SCH) soil layers. In each case, the mean of the soil water storage data from the corresponding soil depth, region, and time step was used as explanatory variable. Convergence of the models occurred at approx. 2,000 updates. Convergence and quality assessment, “burn-in” iterations (10,000), and further 10,000 iterations were performed as described in Section 2.2.1. The model for soil water storage performed well in all three regions and both soil layers (see supporting information).

2.3 Soil water balance model for water fluxes

We used a soil water balance model, developed to calculate vertical soil water fluxes (in $\text{mm} = \text{L m}^{-2}$) from two soil layers in grassland (Kreutziger, 2006; Leimer et al., 2014) based on DVWK (1996). The model was successfully applied in several studies (Leimer et al., 2014; Oelmann, Kreutziger, Temperton, et al., 2007; Oelmann, Kreutziger, Bol, & Wilcke, 2007; Sprenger et al., 2013) and well validated against the numeric models Hydrus-1D and WAVE, which are based on the Richards equation (Kreutziger, 2006; Sprenger et al., 2013). The soil water balance model is based on the soil water balance equation, separated into two soil layers (1 = topsoil, 2 = subsoil; Eq. 6 and 7)

$$I_1 + UF_1 = DF_1 + ETa_1 + \Delta S_1, \text{ with } I_1 = P \quad (6)$$

$$I_2 + UF_2 = DF_2 + ETa_2 + \Delta S_2, \text{ with } I_2 = NF_1 = DF_1 - UF_1 \quad (7)$$

where I is the water input into the soil from above, i.e. precipitation (P) in the case of topsoil and net flux (NF) of the topsoil in the case of subsoil, UF is upward flux, DF is downward flux, ETa is actual evapotranspiration, and ΔS is the change in soil water storage between two subsequent observation dates ($\Delta S = S_{t_2} - S_{t_1}$). UF is defined as the upward water flux via capillary rise which does not include water transported by roots.

The employed water balance model is described in Leimer et al. (2014). Briefly, it consists of the Eq. 8-10.

$$\text{if } I - \Delta S < 0 \text{ then } ETa = 0, DF = 0, UF = \Delta S - I \quad (8)$$

$$\text{if } I - \Delta S \leq r \cdot ETp \text{ then } ETa = I - \Delta S, DF = 0, UF = 0 \quad (9)$$

$$\text{if } I - \Delta S > r \cdot ETp \text{ then } ETa = r \cdot ETp, DF = I - \Delta S - r \cdot ETp, UF = 0 \quad (10)$$

Potential evapotranspiration (ETp) is used as upper limit of total ETa . The percentage of roots (r) in each layer is used as a proxy for the percentage of potential evapotranspiration that could be evapotranspired from the respective layer (Eq. 9 and 10). The model assumes that there is no lateral flow, which is reasonable at our mostly flat study sites. If the boundary conditions of Eq. 8 apply, it is possible that ETa and UF are underestimated at the fortnightly resolution. If the boundary conditions of Eq. 9 apply, it is possible that additional water, originating from below the subsoil layer, undergoes evapotranspiration. This would also cause an underestimation of ETa . Therefore, we aggregated our data to annual values and evaluated the simulated ETa with data provided by the DWD Climate Data Center (2015).

Because of the shallow soils in the ALB, only water fluxes in topsoil (0-0.15 m) were simulated for the 9 plots in this region. In HAI and SCH, we applied the model to topsoil (0-0.15 m) and subsoil (HAI: 0.15-0.35 m, SCH: 0.15-0.70 m). We calculated biweekly water fluxes (mm (14 days)^{-1}) for each depth layer and plot using the preprocessed soil water storage and potential evapotranspiration data and subsequently calculated annual fluxes (mm year^{-1}) from 2010 to 2015.

2.4 Statistical analyses

Annual water fluxes (ETa , DF , and UF) were analyzed for plant diversity effects using repeated measures ANOVAs (type I). In each ANOVA, we accounted for the effects of region (ALB, HAI, or SCH) and land-use class (pasture, mown pasture, or meadow) before any plant diversity variable. The following plant diversity variables were included in separate ANOVAs after region and land-use class: species richness, cover of grasses, cover of herbs, cover of legumes, number of grasses, number of herbs, and number of legumes. Additionally, we analyzed the effect of aboveground biomass on water fluxes by including biomass after region and land-use in the repeated-measures ANOVAs. Residuals were checked graphically for compliance with the requirements of ANOVA. All statistical analyses were done with the R 3.2.0 software package (R Core Team, 2015). The ANOVAs were performed with the function `aov()`.

3 Results

Modeled annual ETa (sum of topsoil and subsoil, Fig. 1, Tab. S1) agreed well with long-term mean (1991-2010) ETa of grass growing on sandy loam, provided by the Deutscher Wetterdienst (DWD) per region. The closest climate station per study region which was located at a similar elevation as our study plots and where ETa data was available was the station Münsingen-Apfelstetten (ID: 3402) with 471.6 mm ETa for the region ALB, the station Leinefelde (ID: 2925) with 431.9 mm for HAI, and the station Angermünde (ID: 164) with 409.1 mm for SCH.

Of the three regions, ALB had the highest P and DF and lowest ETp (Fig. 1). UF into subsoil was highest in SCH, whereas there was almost no UF into topsoil in ALB (Fig. 1, Tab. S1). All water fluxes from topsoil significantly differed between regions (Tab. 1). Land-use class did not significantly affect any of the water fluxes (Tab. 1). Likewise, land-use intensity did not significantly affect any of the water fluxes except for ETa from topsoil (Tab. S3). All water fluxes varied significantly between years (Tab. 1).

ETa from topsoil significantly decreased with increasing species richness in all three regions, while ETa from subsoil increased significantly in SCH and HAI (Tab. 1, Fig. 2a, b). DF from

topsoil increased marginally significantly ($p < 0.10$) with species richness, independent of analyzing the data of only SCH and HAI or of all three regions together (Tab. 1). The other water fluxes (DF from subsoil and UF from topsoil and subsoil) were not significantly related with species richness. Species richness was negatively correlated with biomass (Socher et al., 2012, Fig. S2). Accordingly, the relationship between biomass and ETa from topsoil and subsoil was opposed to that of species richness (Tab. S4). Furthermore, DF from subsoil significantly increased with increasing biomass (Tab. S4).

Cover of herbs and legumes did not show any significant relationship with water fluxes. Only the negative within-subject effect (i.e. the annual variation on the same plot) of cover of grasses on UF into topsoil was significant, if the data of all three regions was analyzed together (Tab. 2). The comparison between plots indicated that ETa from topsoil decreased at least marginally significantly with increasing numbers of grasses, legumes, and herbs (Tab. 2, Fig. 2c, e, g). When we considered the annual variation from 2010 to 2015 on the same plots (the within-subject effect), we found a positive relationship between number of grasses and ETa from topsoil (Tab. 2, Fig. 3a). In subsoil, ETa significantly increased with increasing numbers of herbs and legumes (Tab. 2, Fig. 2d,f). DF from topsoil significantly increased with increasing number of grasses (Tab. 2, Fig. 4). When we considered the annual variation of UF into topsoil on each plot, we observed a positive relationship with the number of herbs (Fig. 3b) and a negative relationship with the number of grasses, if the data of all regions was analyzed together (Tab. 2).

The sum of annual ETa from topsoil and subsoil (for the region ALB, ETa from subsoil was assumed to be 0 mm) was not significantly related with species richness, biomass, and cover and number of grasses, legumes, and herbs, respectively. Only the within-subject effect (i.e. the annual variation from 2010 to 2015) of the number of grasses showed a significant increase of summed ETa from topsoil and subsoil with increasing number of grasses ($F = 6.68$, $p = 0.011$). Region and land-use class did not significantly affect summed ETa , but summed ETa varied significantly between years ($F = 5.95$, $p < 0.001$).

4 Discussion

Mean annual ETa per region agrees well with long-term mean ETa provided by the Deutscher Wetterdienst (DWD) and the observed differences in water fluxes between study regions are in accordance with the differences in climate and geological setting. ALB is situated at the highest studied elevation and has the lowest annual mean temperature of the three regions and therefore showed the highest P and lowest ETp (Fig. 1). The calcareous bedrock with karst phenomena and the shallow and stone-rich soils in ALB are, together with the high P and low ETp , the reason for the high DF and very low UF (Fig. 1). In contrast, SCH, which is situated at the lowest elevation and has the highest annual mean temperature, showed the lowest P and highest ETp . According to the low P , ETa from topsoil was lowest in SCH. The deeper soil layers in SCH are influenced by groundwater, which is reflected by the high UF into subsoil, which increases water supply for plants and results in the high ETa from subsoil (Fig. 1).

4.1 Land-use class

In contrast to our hypothesis, water fluxes were not affected by land-use class (Tab. 1). If land-use intensity as continuous variable instead of land-use class as categorical variable was considered, ETa from topsoil was the only water flux that significantly increased with increasing land-use intensity (Tab. S3). This can likely be explained by increasing biomass with increasing land-use intensity. The absence of a land-use class effect is in accordance with the findings of Fatichi et al. (2014) that only unsustainable management like very low

grass cuts or heavy grazing modified the water balance of subalpine grasslands in Switzerland and moderate management did not have a detectable effect. Consequences of the land-use measures grazing, mowing, and fertilization (e.g. modification of biomass, soil compaction), which might affect the water balance by changing transpiration, infiltration rates or soil evaporation, were either too weak or neutralized each other in the land-use classes of our study.

4.2 Plant species richness

While in manipulative biodiversity experiments decreasing ETa from topsoil with increasing species richness (Fig. 2a) was attributed to higher biomass and thus more shading of the soil and more dew formation in species-rich mixtures (Caldeira et al., 2001; Rosenkranz, Wilcke, Eisenhauer, & Oelmann, 2012; Spehn, Joshi, Schmid, Alpehi, & Körner, 2000), this explanation does not hold true for our study because species richness was negatively correlated with biomass (Socher et al., 2012, Fig. S2). Moreover, ETa from topsoil increased with increasing biomass in our study (Tab. S4). In several studies, ETa increased with biomass because of increased transpiration and interception loss (De Boeck et al., 2006; Inauen et al., 2013; Obojes et al., 2015; Verheyen et al., 2008). This likely explains the detected positive biomass- and negative species richness- ETa relationship in topsoil (Fig. 2a), given that species richness and biomass are negatively correlated in agriculturally managed Central European grasslands (Socher et al., 2012, Fig. S2). Because of this correlation between biomass and species richness, their separate effects cannot be disentangled. As biomass did not significantly affect summed ETa from topsoil and subsoil in our study (Tab. S4), alternative explanations should also be considered. The detected species richness- ETa relationship might be caused by a higher infiltration capacity in species-rich plant mixtures. Such a relationship has been reported by C. Fischer et al. (2015), who attributed it to increased soil structural stability below species-rich compared to species-poor plant mixtures, which were all grown under homogeneous soil, topography, and management conditions. A higher infiltration capacity would cause the water to percolate faster to deeper soil layers, thereby reducing the possibility for water uptake and subsequent transpiration by plants from topsoil. More deep-reaching roots in species-rich plant communities (Mueller, Tilman, Fornara, & Hobbie, 2013) might also explain the negative species richness- ETa relationship in topsoil, as deep roots facilitate water uptake from subsoil and subsequently might reduce plant water uptake and subsequent transpiration from topsoil. The finding of Klaus et al. (2016) that drought stress was lower under higher plant diversity further points toward a better exploitation of water resources via deeper roots in species-rich grasslands. A higher infiltration capacity and deeper roots in species-rich mixtures would furthermore explain the increasing ETa with species richness in subsoil (Fig. 2b). The fact that DF from topsoil to subsoil increased marginally significantly with species richness (Tab. 1) supports the hypothesis that a higher infiltration capacity in species-rich mixtures might have contributed to reducing ETa from topsoil and increasing ETa from subsoil in species-rich mixtures.

The finding that summed ETa from topsoil and subsoil was not related with species richness is in accordance with findings from some manipulative biodiversity experiments (Leimer et al., 2014; Stocker, Körner, Schmid, Niklaus, & Leadley, 1999). Furthermore, Obojes et al. (2015) reported that ETa from mountain grasslands, where biodiversity was not manipulated, was not related with species richness in two of three studied regions (in France and Austria). Only in the third region (in Switzerland), ETa increased with species richness, like in the manipulative biodiversity experiments of De Boeck et al. (2006) and Verheyen et al. (2008). However, it is not clear, if the complete rooting zone was investigated in all studies. Therefore, we assume that, if ETa , DF , and UF from the complete rooting zone are

considered, species richness has no influence on the water balance. At the same time, evidence accumulates that species-rich mixtures exploit different soil depths for water uptake than species-poor mixtures (Caldeira et al., 2001; Leimer et al., 2014) without changing the overall water balance (Tab. 1). Such differences in the exploited soil depths would explain differing results in previous studies.

4.3 Cover and number of species in plant functional groups

Except for a significant within-subject effect (describing the effect of annual variation on the same plot) of the cover of grasses on UF , cover of grasses, legumes, and herbs showed no relationship with any of the studied water fluxes. In contrast, the numbers of grass, legume, and herb species were significantly related with several of the studied water fluxes. The detected relationships between number of species in individual functional groups with ETa in the between-plot comparisons mainly correspond to the detected relationships with total species richness (Fig. 2, Tab. 1, 2). This can be attributed to a close correlation between total species richness and the number of grass, legume, and herb species, respectively.

Plots with more grass species had a lower ETa from topsoil than plots with less grass species, while ETa from subsoil was not affected by the number of grasses (Fig. 2g, Tab. 2). The opposite would have been expected according to the findings of Obojes et al. (2015) that the abundance of tall grass species increased ETa and of Leimer et al. (2014) that the presence of grasses increased ETa from topsoil and decreased ETa from subsoil. In contrast to the between-subject comparison, the within-subject comparison showed a significant positive relationship between number of grasses and ETa from topsoil (Tab. 2). This means that ETa from topsoil on an individual plot was higher in years with more grass species on this plot (Fig. 3a). This within-subject effect is in accordance with the findings of Obojes et al. (2015) in mountain grasslands and Leimer et al. (2014) for plots in a manipulative biodiversity experiment with homogeneous soil, topography, and management. As properties of soil and topography vary between plots but not within plots in our study, the opposite between-subject effect is likely caused by differences in these properties between the studied plots. The finding that DF from topsoil to subsoil increases with the number of grass species (Fig. 4) might point towards a higher infiltration capacity in plots with more grasses as discussed in the previous section for species richness. However, C. Fischer et al. (2015) did not detect a significant effect of the presence/absence of grasses on infiltration capacity in a manipulative biodiversity experiment.

The decrease of ETa from topsoil and increase of ETa from subsoil with increasing number of legumes (only in HAI and SCH; Fig. 2e, f) complies with findings from a manipulative biodiversity experiment (Leimer et al., 2014). The latter authors explained this relationship by less root biomass in topsoil in mixtures with legumes than in mixtures without legumes (Bessler et al., 2012) and a higher biomass production of legumes-containing mixtures (Marquard et al., 2009) that increased shading (Spehn, Joshi, Schmid, Alpei, & Körner, 2000), decreased soil evaporation and subsequently ETa from topsoil. Both explanations are based on the fact that in a nitrogen-limited system (e.g., unfertilized grassland, managed with low intensity), legumes increase the nitrogen availability for all plants in the mixture by their ability to fix N_2 from the atmosphere. This additional nitrogen supports biomass production and facilitates the growth of deeper roots, which allow for acquisition of additional resources, including water, from deeper soil layers. In the study of Obojes et al. (2015), the direction and the significance of the relationship between legumes and ETa varied between regions. The differing results per region, also found for ALB (shallow soils), might have been caused by differences in soil and root properties (main rooting zone, rooting depth, root distribution etc.). In contrast to the finding from a manipulative biodiversity experiment (Leimer et al.,

2014), DF from topsoil to subsoil did not show a significant positive relationship with legumes.

The number of herb species showed the same relationship with ETa from topsoil and subsoil as species richness (Fig. 2c, d), which disagrees with the results of Leimer et al. (2014) from a manipulative biodiversity experiment. However, a comparison of their results with this study is difficult because of differences in soil properties and because in the study of Leimer et al. (2014) herbs were separated into small and tall herbs, which had different relationships with water fluxes, and their effects varied over time (seasonally and with time since establishment of the experiment). Especially, the taproot system of tall herbs might facilitate water use from deeper soil layers (Leimer et al., 2014) and possibly led to increased ETa from subsoil in our study (Fig. 2d).

5 Conclusions

(1) In contrast to our first hypothesis, water fluxes from topsoil and subsoil did not significantly vary between the land-use classes pasture, mown pasture, and meadow in the six study years.

(2) In accordance with our second hypothesis, we found that plant species richness decreased ETa from topsoil and increased ETa from subsoil. However, this finding cannot be explained by the same processes as in manipulative biodiversity experiments. We therefore hypothesize that a higher infiltration capacity in soil under species-rich mixtures and differences in the soil depths, exploited for resources by differently diverse plant mixtures, contributed to the detected species richness effects on water fluxes.

(3) Against our third hypothesis, the cover of specific plant functional groups did not influence water fluxes considerably. The number of plant species in the functional groups showed several significant relationships with water fluxes. However, a comparison of these results with results from manipulative biodiversity experiments was difficult because of the establishment of artificial treatments in manipulative biodiversity experiments (e.g. presence/absence of grasses) which cannot easily be found in real-world ecosystems.

We conclude that manipulative biodiversity experiments are a useful tool to obtain an idea of what plant biodiversity is capable to perform, but these results need to be confirmed with real-world studies. Our study of 29 real-world ecosystems, indicates that groundwater recharge, controlled by DF from subsoil, and total ecosystem evapotranspiration are not influenced by land-use class or plant species richness. However, the soil depth partitioning of resource use seems to vary between differently diverse plant mixtures, to which future studies should dedicate special attention.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article.

Accepted Article

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Table 1: Results of the repeated-measures analyses of variance (type I) for annual water fluxes [actual evapotranspiration (*ETa*), downward flux (*DF*), upward flux (*UF*), and net flux (*NF = DF - UF*)] between 2010 and 2015 in the studied regions Schwäbische Alb (ALB), Hainich-Dün (HAI), and Schorfheide-Chorin (SCH) from topsoil (0-0.15 m) and subsoil (HAI 0.15-0.35 m, SCH: 0.15-0.70 m). Significant p-values ($p < 0.05$) are given in bold, marginally significant p-values ($p < 0.10$) in italics. Arrows indicate positive (\uparrow) and negative (\downarrow) effects, respectively.

	df	SS	ETa		SS	DF		SS	UF		SS	NF					
			F	p		F	p		F	p		F	p				
<i>Between-subject effects</i>																	
ALB, HAI & SCH	region	2	633309	17.81	<0.001	1038859	12.13	<0.001	806	6.74	0.005	1093841	12.75	<0.001			
	land-use type	2	27260	0.77	0.476	1822	0.02	0.979	119	0.99	0.386	2340	0.03	0.973			
	species richness	1	107768	6.06	0.022	\downarrow	151241	3.53	<i>0.073</i>	\uparrow	27	0.45	0.511	147249	3.43	<i>0.077</i>	\uparrow
	residuals	23	408880			984944			1376			986648					
	<i>Within-subject effects</i>																
	year	5	59724	5.93	<0.001	1699241	25.43	<0.001	2681	15.14	<0.001	1696644	24.84	<0.001			
	species richness	1	676	0.34	0.563	5837	0.44	0.510	53	1.50	0.223	4778	0.35	0.556			
	residuals	139	279820			1857965			4924			1898407					
	<i>Between-subject effects</i>																
	HAI & SCH	region	1	160481	10.44	0.006	90470	4.10	<i>0.061</i>	3	0.03	0.856	89440	4.00	<i>0.065</i>		
land-use type		2	28900	0.94	0.413	77224	1.75	0.207	171	0.99	0.396	77266	1.72	0.213			
species richness		1	129506	8.42	0.011	\downarrow	96139	4.36	<i>0.054</i>	\uparrow	5	0.06	0.815	94768	4.22	<i>0.058</i>	\uparrow
residuals		15	230683			330764			1304			337192					
<i>Within-subject effects</i>																	
year		5	17156	3.04	0.014	730638	14.06	<0.001	3160	14.31	<0.001	731729	13.65	<0.001			
species richness		1	69	0.06	0.805	16720	1.61	0.208	87	1.96	0.165	1440	1.34	0.249			
residuals		94	106269			977195			4150			10724					
<i>Between-subject effects</i>																	
subsoil		region	1	153161	7.54	0.015	67779	3.68	<i>0.074</i>	191575	11.53	0.004	487256	34.85	<0.001		
	land-use type	2	22605	0.56	0.585	39056	1.06	0.371	9858	0.30	0.748	13614	0.49	0.624			
	species richness	1	110910	5.46	0.034	\uparrow	4751	0.26	0.619	1527	0.09	0.766	861	0.06	0.804		
	residuals	15	304745			276105			249276			209742					

Within-subject effects

year	5	2862	3.17	0.011	691960	11.87	<0.001	26370	2.94	0.016	641210	11.68	<0.001
species richness	1	83	0.46	0.499	10011	0.86	0.356	588	0.33	0.568	15450	1.41	0.239
residuals	94	16977			1095558			168615			1032467		

Table 2: Results of the repeated-measures analyses of variance (type I) for cover and number of grasses, herbs, and legumes, respectively, if fitted separately after region and land-use class (i.e. if species richness in Table 1 is replaced by one of these explanatory variables). Results refer to annual actual evapotranspiration (*ETa*), downward flux (*DF*), upward flux (*UF*), and net flux ($NF = DF - UF$) between 2010 and 2015 in the studied regions Schwäbische Alb (ALB), Hainich-Dün (HAI), and Schorfheide-Chorin (SCH) from topsoil (0-0.15 m) and subsoil (HAI: 0.15-0.35 m, SCH: 0.15-0.70 m). Significant p-values ($p < 0.05$) are given in bold, marginally significant p-values ($p < 0.10$) in italics. Arrows indicate positive (\uparrow) and negative (\downarrow) effects, respectively.

		df	SS	ETa		SS	DF		SS	UF		SS	NF					
				F	p		F	p		F	p		F	p				
ALB, HAI & SCH	cover grasses	<i>Between-subject effects</i>	1	6527	0.29	0.593	96	0.00	0.965	2	0.04	0.846	68	0.00	0.971			
		<i>Within-subject effects</i>	1	5921	3.00	0.086	8533	0.64	0.425	169	4.88	0.029	\downarrow	6302	0.46	0.498		
	cover herbs	<i>Between-subject effects</i>	1	913	0.04	0.842	52032	1.10	0.304	6	0.11	0.747	50878	1.08	0.309			
		<i>Within-subject effects</i>	1	568	0.28	0.596	1097	0.08	0.775	0	0.00	0.952	1073	0.08	0.780			
	cover legumes	<i>Between-subject effects</i>	1	20108	0.93	0.345	73049	1.58	0.221	2	0.03	0.859	73809	1.60	0.218			
		<i>Within-subject effects</i>	1	505	0.25	0.617	2420	0.18	0.671	12	0.34	0.559	2088	0.15	0.697			
	topsoil number grasses	<i>Between-subject effects</i>	1	67597	3.46	<i>0.076</i>	\downarrow	202609	4.99	0.035	\uparrow	44	0.75	0.394	196649	4.83	0.038	\uparrow
		<i>Within-subject effects</i>	1	12107	6.27	0.013	\uparrow	8758	0.66	0.419	149	4.30	0.040	\downarrow	6621	0.49	0.487	
	number herbs	<i>Between-subject effects</i>	1	100159	5.53	0.028	\downarrow	120144	2.72	0.113	22	0.36	0.552	116921	2.64	0.118		
		<i>Within-subject effects</i>	1	3528	1.77	0.186		16219	1.22	0.271	195	5.68	0.019	\uparrow	12854	0.95	0.333	
	number legumes	<i>Between-subject effects</i>	1	65457	3.34	<i>0.081</i>	\downarrow	28216	0.59	0.452	1	0.01	0.914	27930	0.58	0.454		
		<i>Within-subject effects</i>	1	10218	5.26	0.023	\downarrow	4410	0.33	0.567	40	1.12	0.292	3612	0.26	0.608		
HAI & SCH	cover grasses	<i>Between-subject effects</i>	1	4376	0.18	0.674	1312	0.05	0.833	7	0.08	0.787	1133	0.04	0.845			
		<i>Within-subject effects</i>	1	187	0.17	0.685	3925	0.37	0.543	26	0.58	0.449	3313	0.31	0.582			
	cover herbs	<i>Between-subject effects</i>	1	1062	0.04	0.836	33951	1.30	0.273	4	0.04	0.838	33237	1.25	0.281			
		<i>Within-subject effects</i>	1	78	0.07	0.794	2310	0.22	0.641	25	0.55	0.459	2813	0.26	0.612			
	cover legumes	<i>Between-subject effects</i>	1	6153	0.26	0.617	1616	0.06	0.815	1	0.01	0.906	1528	0.05	0.821			
		<i>Within-subject effects</i>	1	63	0.06	0.815	448	0.04	0.837	5	0.12	0.729	355	0.03	0.857			
	topsoil number grasses	<i>Between-subject effects</i>	1	90028	5.00	0.041	\downarrow	100475	4.62	0.048	\uparrow	21	0.25	0.626	97574	4.38	<i>0.054</i>	\uparrow
		<i>Within-subject effects</i>	1	5412	5.04	0.027	\uparrow	2766	0.26	0.610	61	1.37	0.244	2005	0.19	0.668		
	number herbs	<i>Between-subject effects</i>	1	110887	6.67	0.021	\downarrow	67344	2.81	0.114	1	0.01	0.907	66769	2.74	0.119		
		<i>Within-subject effects</i>	1	764	0.68	0.412		30611	2.99	<i>0.087</i>	\uparrow	214	5.00	0.028	\uparrow	25708	2.45	0.123
	number legumes	<i>Between-subject effects</i>	1	108393	6.46	0.023	\downarrow	78982	3.41	<i>0.085</i>	\uparrow	1	0.01	0.932	78530	3.33	<i>0.088</i>	\uparrow
		<i>Within-subject effects</i>	1	0	0.00	0.992		2784	0.26	0.609	16	0.35	0.556	2382	0.22	0.641		
subsoil cover grasses	<i>Between-subject effects</i>	1	15609	0.59	0.456		33706	2.05	0.173	570	0.03	0.856	25511	2.07	0.171			
	<i>Within-subject effects</i>	1	170	0.94	0.334		4779	0.41	0.524	3212	1.82	0.181	15827	1.44	0.233			
cover herbs	<i>Between-subject effects</i>	1	14749	0.55	0.469		2955	0.16	0.695	11496	0.72	0.409	2794	0.20	0.660			
	<i>Within-subject effects</i>	1	9	0.05	0.828		16443	1.42	0.237	238	0.13	0.717	20637	1.89	0.173			

cover legumes	<i>Between-subject effects</i>	1	12422	0.46	0.507		36091	2.21	0.158		12994	0.82	0.380		5774	0.42	0.525
	<i>Within-subject effects</i>	1	8	0.05	0.830		50	0.00	0.948		1349	0.76	0.387		880	0.08	0.779
number grasses	<i>Between-subject effects</i>	1	61169	2.59	0.128		15	0.00	0.978		2860	0.17	0.683		3291	0.24	0.633
	<i>Within-subject effects</i>	1	100	0.56	0.458		4629	0.40	0.531		1371	0.77	0.383		962	0.09	0.770
number herbs	<i>Between-subject effects</i>	1	97725	4.61	0.049	↑	5420	0.30	0.595		303	0.02	0.895		3160	0.23	0.640
	<i>Within-subject effects</i>	1	7	0.04	0.845		29096	2.54	0.114		2	0.00	0.974		29562	2.73	0.102
number legumes	<i>Between-subject effects</i>	1	113270	5.62	0.032	↑	15591	0.88	0.363		3939	0.24	0.632		3857	0.28	0.605
	<i>Within-subject effects</i>	1	165	0.92	0.341		1052	0.09	0.765		1224	0.69	0.410		7	0.00	0.981

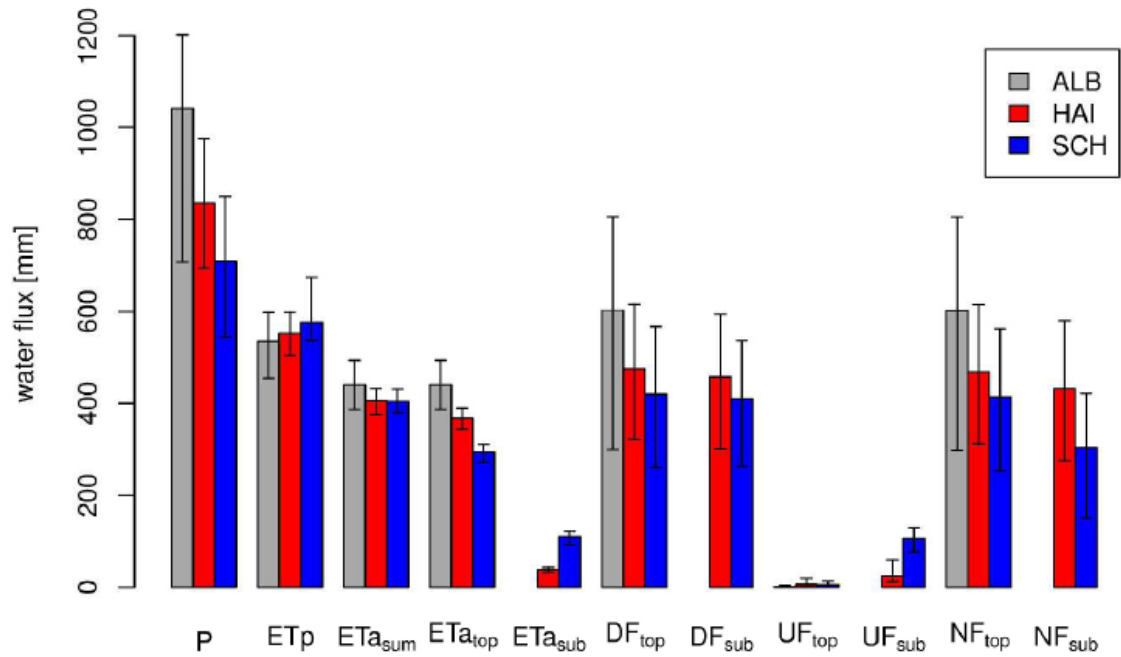


Figure 1: Annual precipitation (P), potential evapotranspiration (ET_p), actual evapotranspiration (ET_a), downward flux (DF), upward flux (UF), and net flux ($NF = DF - UF$) between 2010 and 2015 for each of the study regions Schwäbische Alb (ALB), Hainich-Dün (HAI), and Schorfheide-Chorin (SCH). Annual averages were calculated per region using annual data from 9 plots (SCH: 11 plots) per region. From these averages, mean (box), minimum, and maximum (error bars) values over time were calculated per region. Top refers to the topsoil (0-0.15 m), sub to the subsoil (0.15-0.35 m (HAI) and 0.15-0.70 m (SCH)), and sum to summed ET_a from topsoil and subsoil.

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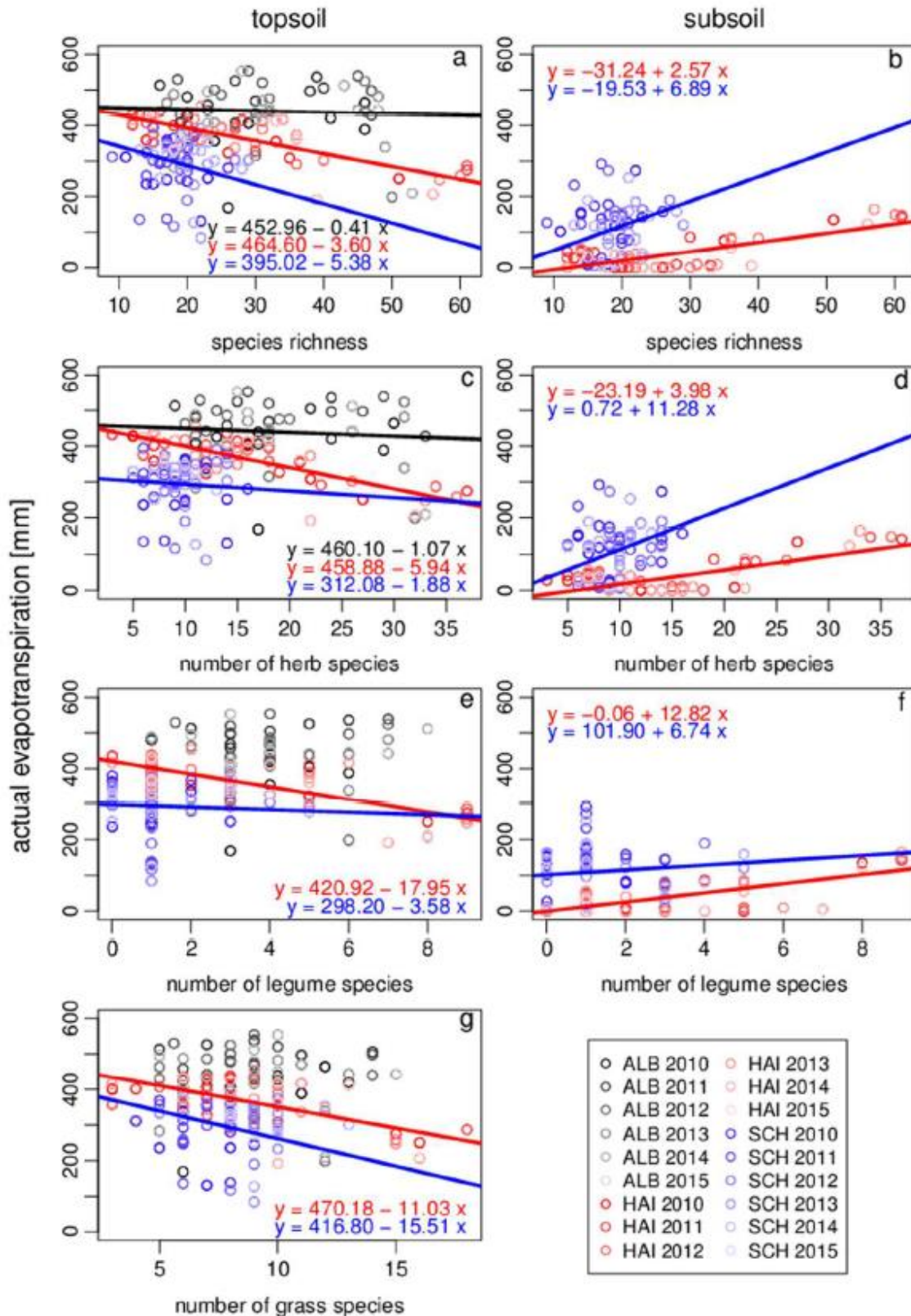


Figure 2: Relationship of species richness (a, b), number of herb species (c, d), number of legume species (e, f), and number of grass species (g) with the annual sum of actual evapotranspiration (ET_a) from topsoil (a, c, e, g) and subsoil (b, d, f) for each study region (ALB: Schwäbische Alb (black), HAI: Hainich-Dün (red), SCH: Schorfheide-Chorin (blue)).

Circles indicate the values per plot and year (2010-2015). Lines and equations represent the linear regression of mean *ETa* per plot on mean species richness, number of herb, legume, and grass species, respectively, per plot during the six studied years. Regressions are only shown for significant between-subject effects in the ANOVA results (Tab. 1, 2).

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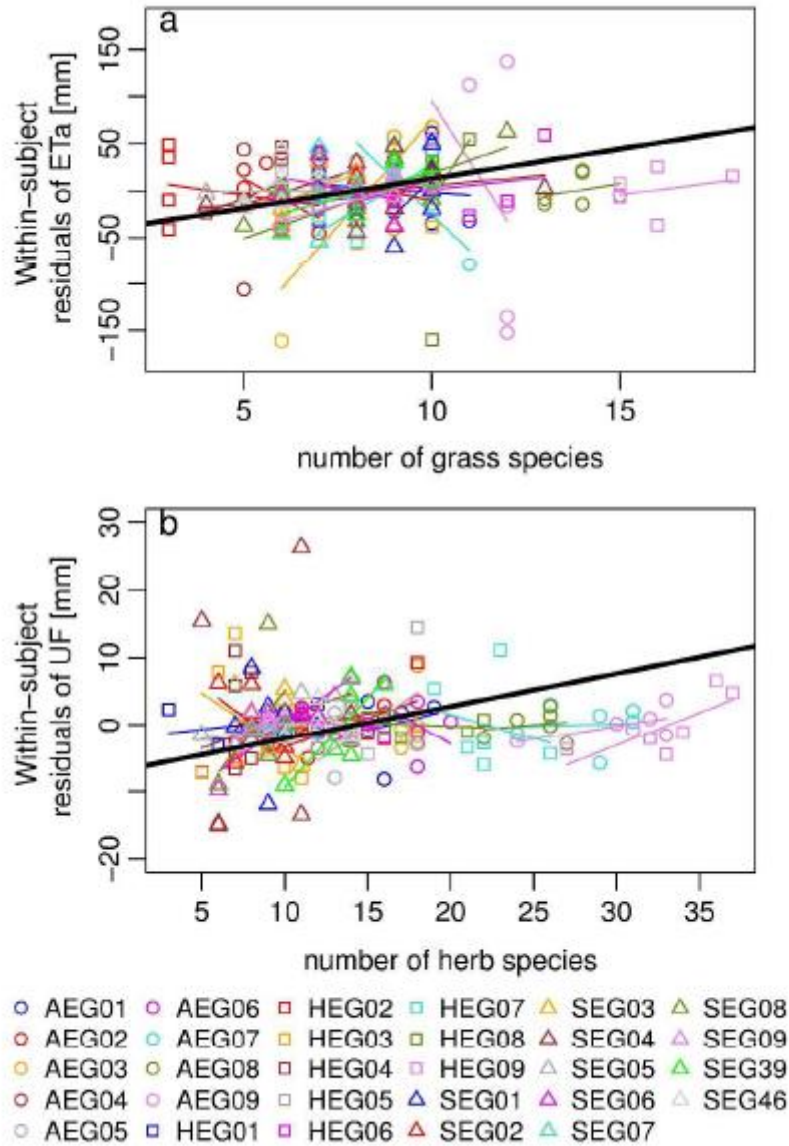


Figure 3: (a) Number of grass species versus within-subject residuals of actual evapotranspiration (ETa) from and (b) number of herb species versus within-subject residuals of upward flux (UF) into topsoil per plot and year, representing the significant within-subject effects in the ANOVA results, which reflect the temporal variation from 2010 to 2015 (Tab. 2). Residuals were obtained after accounting for the effects of region, land-use class, and year in the repeated measures ANOVA. Circles indicate values from grassland plots in the region Schwäbische Alb (AEG01-09), squares values from Hainich-Dün (HEG01-09), and triangles values from Schorfheide-Chorin (SEG01-09, SEG39, SEG46). The black line indicates the mean slope and intercept of the regressions of within-subject residuals of ETa on number of grass species or within-subject residuals of UF on number of herb species per individual plot (colored lines) using data from six years (2010-2015).

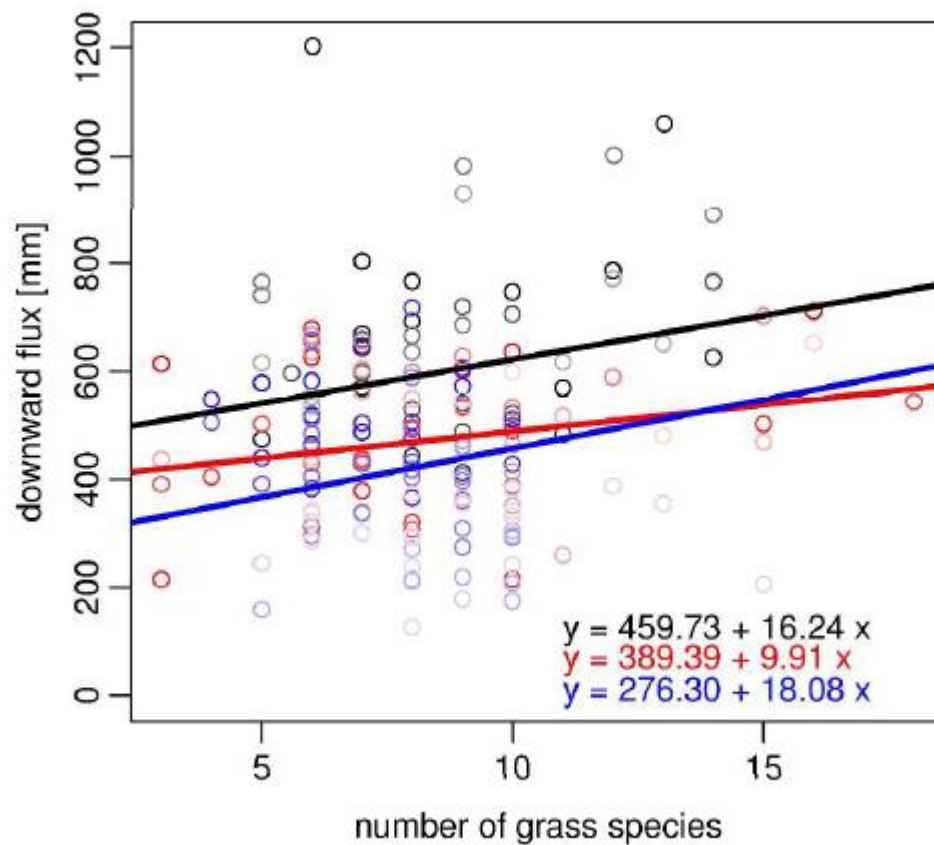


Figure 4: Relationship between number of grass species and downward water flux from topsoil for each study region (ALB: Schwäbische Alb (black), HAI: Hainich-Dün (red), SCH: Schorfheide-Chorin (blue)). Circles indicate the values per plot and year (2010-2015). Lines and equations represent the linear regression of mean *DF* per plot on mean number of grass species per plot during the six studied years. For the legend see Fig. 2.