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## **Plant diversity enhances production and downward transport of biodegradable dissolved organic matter**

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## Abstract

1. Plant diversity is an important driver of belowground ecosystem functions, such as root growth, soil organic matter (SOM) storage, and microbial metabolism, mainly by influencing the interactions between plant roots and soil. Dissolved organic matter (DOM), as the most mobile form of SOM, plays a crucial role for a multitude of soil processes that are central for ecosystem functioning. Thus, DOM is likely to be an important mediator of plant diversity effects on soil processes. However, the relationships between plant diversity and DOM have not been studied so far.
2. We investigated the mechanisms underlying plant diversity effects on concentrations of DOM using continuous soil water sampling across 6 years and 62 plant communities in a long-term grassland biodiversity experiment in Jena, Germany. Furthermore, we investigated plant diversity effects on the molecular properties of DOM in a subset of the samples.
3. Although DOM concentrations were highly variable over the course of the year with highest concentrations in summer and autumn, we found that DOM concentrations consistently increased with plant diversity across seasons. The positive plant diversity effect on DOM concentrations was mainly mediated by increased microbial activity and newly sequestered carbon in topsoil. However, the effect of soil microbial activity on DOM concentrations differed between seasons, indicating DOM consumption in winter and spring, and DOM production in summer and autumn. Furthermore, we found increased contents of small and easily decomposable DOM molecules reaching deeper soil layers with high plant diversity.
4. *Synthesis.* Our findings suggest that plant diversity enhances the continuous downward transport of DOM in multiple ways. On the one hand, higher plant diversity results in higher DOM concentrations, on the other hand, this DOM is less degraded. The present study indicates, for the first time, that higher plant diversity enhances the downward transport of dissolved molecules that likely stimulate soil development in deeper layers and therefore increase soil fertility.

## Keywords:

Biodiversity, Decomposition, Dissolved organic carbon, Ecosystem functions and services, Plant–soil interactions, Subsoil, Vegetation

## Introduction

The loss of biodiversity (Barnosky *et al.* 2011) has severe consequences for multiple ecosystem functions, such as reduced plant biomass production, SOM storage, and soil nutrient provision (Balvanera *et al.* 2006; Hooper *et al.* 2012). Among other factors, the functioning of terrestrial ecosystems depends on the materials and energy supplied by plants, the retention of nutrients in the soil, and the provision of nutrients to plants by microbial mineralisation of organic matter to a great extent (Bardgett & van der Putten 2014; Wagg *et al.* 2014). Plant diversity has been demonstrated to impact plant-soil interactions *via* the release of increased quantities or more diverse rhizodeposits in the soil (Eisenhauer *et al.* 2017; Lange *et al.* 2019) or by enhancing access of the microbial community to the rhizodeposits (Mellado-Vazquez *et al.* 2016). In turn, with higher plant diversity more active soil microbial communities mineralize higher quantities of organic matter (Zak *et al.* 2003; Eisenhauer *et al.* 2010), and thereby provide more plant-available nutrients (Oelmann *et al.* 2011; Hacker *et al.* 2015; Lange *et al.* 2019). These plant-soil interactions mostly occur in the liquid phase of soil (Schimel & Weintraub 2003) and are mediated by dissolved organic molecules, which are detected in DOM. However, despite this important function, the role of DOM in the relationship between plant diversity and soil functions has scarcely been addressed.

In a previous study in the Jena Experiment, a positive long-term effect of plant species richness on annual average concentrations of dissolved organic carbon (henceforth termed DOM concentrations) below the densest rooting zone of the top 30 cm was found (Lange *et al.* 2019). However, this increase due to plant diversity was only observed several years after the experiment had been established. The time lag of the plant diversity effect was mainly attributed to a gradual change in soil conditions, as plant diversity-dependent accumulation of organic matter in the topsoil takes time (Lange *et al.* 2015; Lange *et al.* 2019). A similar trend of enhanced accumulation of SOM in high-diversity communities was also reported from other biodiversity experiments in grasslands (Fornara & Tilman 2008; Cong *et al.* 2014) and forest (Li *et al.* 2019). However, the mechanisms underlying the plant diversity effect on DOM are not yet understood.

Generally, DOM is generated by decomposition of dead organic material or exudation of organic substances by roots (Evans, Monteith & Cooper 2005). The quality and quantity of root inputs as well as the soil carbon stocks depend on plant community composition and diversity (Hooper *et al.* 2000; Steinbeiss *et al.* 2008; De Deyn, Quirk & Bardgett 2011; Eisenhauer *et al.* 2017; Lange *et al.* 2019). Thus, changes in plant diversity are likely to impact DOM dynamics. In the upper soil, DOM is mostly plant-derived. However, plant-derived DOM compounds rapidly decline with depth (Scheibe, Krantz & Gleixner

2012; Klotzbücher *et al.* 2016), as DOM is subject to microbial uptake and transformation, as well as to sorption processes during its downward movement through the soil profile (Kaiser & Kalbitz 2012; Roth *et al.* 2019). At the same time, soil microbial community composition and metabolic activity are strongly influenced by plant diversity (Zak *et al.* 2003; Eisenhauer *et al.* 2010; Lange *et al.* 2014; Schmid *et al.* 2019). However, the microbial impact on DOM may be contradictory. Besides the consumption of DOM and the accompanied decline in DOM concentration, increased microbial activity can also enhance mineralization of SOM, thereby increasing DOM concentrations due to more residues of solubilized organic molecules (discussed in Kalbitz *et al.* 2000). Increases or decreases in DOM concentrations can indicate whether the microbial community acts as a sink or source of DOM (Neff & Asner 2001). Furthermore, microbial cycling of DOM impacts both its concentration and molecular properties (Roth *et al.* 2019). As a result of the microbial cycling, the more processed DOM is assumed to be both less bioavailable and less biodegradable for further microbial processing (Marschner & Kalbitz 2003; Don, Roedenbeck & Gleixner 2013). This has consequences for the microbial communities subsequently exposed to the DOM during its downward transport in soil (Leinemann *et al.* 2018). However, the effect of plant diversity on the microbial DOM production and consumption patterns as well as on the molecular properties of DOM is unclear.

Furthermore, the interplay between soil microorganisms and DOM is highly sensitive to environmental conditions, such as seasonal fluctuations in temperature and soil moisture (Kalbitz *et al.* 2000). In particular, DOM concentrations are subject to strong seasonal variations with the highest concentrations in late summer (Don & Schulze 2008). Moreover, a shift in plant-soil interactions during the course of the growing season has been reported (Eisenhauer *et al.* 2018), suggesting that the first half of the growing season is presumably dominated by plant inputs of rhizodeposits, whereas the second half is dominated by decomposition of more slowly decomposable dead plant residues (Kuzyakov 2002). However, so far it is unclear whether plant diversity affects DOM concentrations differently during the course of the year and, if so, what the drivers are in different seasons. Identifying the drivers among seasons might give detailed insights into the mechanisms that underlie plant diversity effects on plant-soil interactions with broad implications for the understanding of soil functioning.

Here, we investigated how plant diversity influences DOM concentrations in the Jena Experiment, and whether or not plant diversity impacts the molecular properties of DOM. To explore the mechanisms underlying potential plant diversity effects, we tested the impact of known drivers, such as belowground productivity, microbial activity, SOM content, soil texture, and hydrological conditions, on DOM concentrations. We hypothesise that plant diversity increases DOM concentrations, reflecting higher

biological activity, and that this is mainly driven by greater belowground root inputs and accumulated SOM that stimulate the microbial community activity. Therefore, during the growing season, DOM concentrations are highest and the effects of plant diversity on it are strongest. We further hypothesise that plant diversity effects will be reflected in molecular DOM data. In particular, we expect an increase in plant diversity to be reflected by an increase in the amount of plant-derived inputs with a low level of decomposition.

## Methods

### Study site – the Jena Experiment

The study was carried out in the Jena Experiment, a large-scale grassland diversity experiment on the floodplain of the Saale River near the city of Jena (Thuringia, Germany; 50°57'N, 11°35'E; Roscher *et al.* 2004; Weisser *et al.* 2017). The soil of the field site is classified as Eutric Fluvisol. In spring 2002, 82 experimental grassland plots of 20 x 20 m were established. Plots are arranged in four blocks to account for changes in soil characteristics with increasing distance from the river. Soil texture in the upper 30 cm of the soil ranges from sandy loam to silty clay with increasing distance from the river. Sand content declines from 50% in the plots close to the river to 5% in plots that are furthest away from the river, while silt content increases from 35% to 70%, respectively. Similar to silt, the clay content increased with increasing distance from the river from 15 to 25%. During the 40 years prior to establishing the experiment, the field site was an arable field with mineral fertilizer input. The initial physico-chemical properties, such as pH (7.1-8.4), SOM content (5-33 g C kg<sup>-1</sup>), and soil nitrogen concentrations (1.0-2.7 g N kg<sup>-1</sup>), varied across the field site and are considered in the block design of the Jena Experiment.

Plant communities were assembled along gradients of plant species richness (PSR; 1, 2, 4, 8, 16 and 60 species) and functional group richness (1, 2, 3, 4) with species randomly chosen from a pool of 60 Arrhenatherion grassland plant species and from the functional groups grasses, legumes, small herbs, and tall herbs. The sown plant functional compositions along the PSR gradient can be found in Appendix 1. Functional group classification was based on morphological, phenological, and physiological traits (Roscher *et al.* 2004). Experimental plots are weeded manually two to three times a year to maintain the target plant community composition. The plots are mown and the mown plant material is removed twice a year in June and September, but not fertilized, which is typical for extensively used hay meadows in Central Europe.

## **Soil water sampling and analysis**

In April 2002, glass suction plates (pore size 1–1.6  $\mu\text{m}$ , 1 cm thickness, 12 cm in diameter; from UMS GmbH, Munich, Germany), were installed in three of the four blocks (N = 62 plots) at a depth of 30 cm to collect soil water. In 2005, glass suction plates were additionally installed at a depth of 20 cm, so that soil water was sampled at two different depths on all 62 plots. The sampling bottles were continuously evacuated using a negative pressure of between 50 and 350 mbar, so that the suction pressure was approximately 50 mbar above the actual soil water tension. As a consequence, only the soil leachate was collected (Scheffer & Schachtschabel 2002). Cumulative soil water was sampled fortnightly, and DOM concentrations were assessed by analysing the dissolved organic carbon concentration with a high TOC elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). All samples were stored at 4°C until measurements and analysed as soon as possible, i.e., within two weeks after sampling.

## **Environmental and plant community associated variables**

### ***Soil organic matter***

A stratified soil sampling was performed on all main plots of the Jena Experiment (N = 82 plots) in April in 2011 and 2014 (Lange *et al.* 2015). In both sampling campaigns, three soil samples were taken per plot (4.8 cm in diameter, 0–30 cm depth) using a split-tube sampler (Eijkelpkamp Agrisearch Equipment, Giesbeek, the Netherlands). The cores were separated into 5 cm layers according to depth in the field and afterwards pooled per layer. The samples were dried (40°C), sieved (2 mm mesh), and milled (6 min, frequency of 30  $\text{s}^{-1}$ ). The soil organic carbon content of the calcareous soil around Jena was assessed by first determining the total carbon concentration of ground samples with an elemental analyser after combustion at 1150°C (varioMax CN elemental analyzer, Elementar Analysensysteme GmbH, Hanau, Germany). Then, inorganic carbon concentration was measured by elemental analysis after removing organic carbon for 16 h at 450°C in a muffle furnace by oxidation. Finally, soil organic carbon (henceforth termed SOM) concentration was calculated from the difference between total and inorganic carbon concentrations (Steinbeiss *et al.* 2008).

### ***Root and shoot standing biomass***

Root standing biomass was sampled on all plots in 2011 and 2014 (for details see Ravenek *et al.* 2014). Three soil cores (3.5 cm) were taken per plot to a depth of 30 cm and pooled before root washing. Root biomass was calculated as g dry mass per  $\text{m}^2$ .

### ***Soil microbial activity***

Annual measurements of basal respiration on soil from all plots annually in late May or early June (Strecker *et al.* 2016) were used as a proxy for soil microbial activity. For this, five soil samples per plot were taken at a depth of 5 cm, pooled and shortly stored at 5°C until basal respiration measurement. The samples were homogenized, sieved (2 mm) to remove larger roots, animals, and stones, and adjusted to a gravimetric soil water content of 25%. After adapting the soil to the measurement temperature of 22°C for 5 days, basal respiration was measured on ~5 g of fresh soil (equivalent to ca. 3.5 g soil dry weight) using an O<sub>2</sub> micro-compensation apparatus (Scheu 1992). The microbial respiratory response was measured at hourly intervals for 24 h at 22 °C. Basal respiration (μL O<sub>2</sub> h<sup>-1</sup> g soil dry mass<sup>-1</sup>) was determined without any addition of substrate and measured as the mean of the O<sub>2</sub> consumption rates 14 to 24 hours after the start of the measurements (for details see Eisenhauer *et al.* 2010). In order to avoid measuring the Birch effect (Birch 1964) i.e., a pulse in microbial activity following a disturbance and rewetting, we only considered the data after 14 h when the respiration rates had stabilised. The basal respiration data used in this study was previously published (Strecker *et al.* 2016), and data from 2011 were used in Lange *et al.* (2015).

### ***Molecular properties of DOM***

The analysis of the effect of plant diversity on the molecular properties of DOM was based on previously published DOM data (Roth *et al.* 2019). Molecular properties of DOM were determined on subsamples of the soil water sampled in block 2 (plot number N = 20) in May 2014. The reduced dataset of the molecular DOM is the result of the sampling design of the former Roth *et al.* (2019) study that was based on a more extensive sampling infrastructure in block 2. Before measurement, soil water subsamples were acidified to pH 2 (HCl, p.a.) and stored at 2 °C until DOM was concentrated and desalted by solid phase extraction (SPE) (Dittmar *et al.* 2008) using Agilent Bond Elute PPL SPE cartridges (1 g). SPE-DOM is a sub-set of the dissolved DOM which includes the most apolar DOM species through to highly polar molecules, but not the smallest polar molecules such as short chain organic acids and free amino acids (Hawkes *et al.* 2016). Due to the same analytical window for all samples, the effects of plant diversity on the metrics of molecular DOM properties can be considered as consistent across all samples. Based on the DOM concentration of each of the samples, the volume of soil water for extraction was adjusted to load 2 mg organic carbon on the columns. After loading the SPE cartridges with samples, the cartridges were rinsed with acidified ultrapure water and dried with nitrogen. The DOM extracts were eluted with methanol. The average extraction efficiency for soil water DOM was 69% on a carbon basis

(SD = 6%). Molecular size of DOM was analysed with FT-ICR-MS measurements. Extract aliquots were diluted to 20 mg L<sup>-1</sup> organic carbon in ultrapure water/methanol (1:1). We used the Bruker Solarix FT-ICR-MS (15 Tesla) at the University of Oldenburg (Germany). Samples were continuously injected into the electrospray ionization (ESI) source with a flow rate of 120 μL h<sup>-1</sup> and an ESI needle voltage of -4 kV in negative ionization mode. 500 single scans with an ion accumulation time of 0.2 s were recorded over a mass range of a mass-to-charge ratio (m/z) 150 to 2000 and added to one spectrum. An in-house mass reference list was used for internal calibration. For statistical analyses, all masses were excluded that were only detected in one measurement and had no reliable signal-to-noise ratio of the maximum of each m/z value ( $s/n_{\text{Max},i}$ ) (for details see Pohlabein & Dittmar 2015 and references therein). Only singly charged ions were considered (Koch & Dittmar 2006). Consequently, the m/z values represent the molecular mass (in dalton) of the detected ions. To study specific formula based characteristics, the weighted mean of m/z ( $m/z_{\text{wm}}$ ) of hydrogen-to-carbon ratios (H/C<sub>wm</sub>) and of oxygen-to-carbon ratios (O/C<sub>wm</sub>) were calculated. Therefore, each measurement as the sum of the product of the individual information (m/z<sub>i</sub>, H/C<sub>i</sub>, O/C<sub>i</sub>) and relative intensity I<sub>i</sub> was divided by the sum of all intensities i.e.:

$$m/z_{\text{wm}} = \text{sum} (m/z_i * I_i) / \sum(I_i) \quad (\text{eqn 1})$$

The  $m/z_{\text{wm}}$  represents each samples' weighted mean of the detected molecular mass. The m/z, H/C (gives information on the saturation), and O/C (gives information on the oxygenation) can be used to describe the molecular properties of DOM (e.g., Roth *et al.* 2019). Furthermore, based on DOM compounds, an index of degradation ( $I_{\text{deg}}$ ) was calculated. Therefore, the normalised intensities of compounds negatively related to degradation ( $I_{\text{deg,neg}}$ : C<sub>21</sub>H<sub>26</sub>O<sub>11</sub>, C<sub>17</sub>H<sub>20</sub>O<sub>9</sub>, C<sub>19</sub>H<sub>22</sub>O<sub>10</sub>, C<sub>20</sub>H<sub>22</sub>O<sub>10</sub>, C<sub>20</sub>H<sub>24</sub>O<sub>11</sub>) and compounds positively related to degradation ( $I_{\text{deg,pos}}$ : C<sub>13</sub>H<sub>18</sub>O<sub>7</sub>, C<sub>14</sub>H<sub>20</sub>O<sub>7</sub>, C<sub>15</sub>H<sub>22</sub>O<sub>7</sub>, C<sub>15</sub>H<sub>22</sub>O<sub>8</sub>, C<sub>16</sub>H<sub>24</sub>O<sub>8</sub>) were calculated as follows (Flerus *et al.* 2012):

$$I_{\text{deg}} = \text{sum}(I_{\text{deg,neg}}) / (I_{\text{deg,neg}} + I_{\text{deg,pos}}) \quad (\text{eqn 2}),$$

where a lower  $I_{\text{deg}}$  indicates less degradation of DOM. Using linear regressions, Flerus *et al.* (2012) identified single mass peaks that were positively and negatively correlated to <sup>14</sup>C of SPE-DOM measurements from 117 oceanic samples. Thereby, the <sup>14</sup>C gives the bulk age of the samples and is thus an indication of the degradation state of DOM. Although this index was developed using aquatic samples, Roth *et al.* (2019) showed that  $I_{\text{deg}}$  works remarkably well on soil samples.

## Statistical analyses

For statistical analyses, the fortnightly sampling data of DOM concentration were averaged and soil water volume summed for each plot among seasons. Seasons are defined as calendric quarters of the year, namely the first quarter (Q1, Jan – Mar) is winter, Q2 (Apr – Jun) is spring, Q3 (Jul – Sep) is summer, and Q4 (Oct – Dec) is autumn. Aggregating data by seasons allowed to account for the seasonal dynamics in DOM concentrations and soil water volume as well as to reduce the high variability among sampling dates. All statistical analyses were conducted with the statistical software R (version 3.3.0; R Development Core Team 2016).

### *Main effects of plant diversity over time*

Linear mixed-effects models (LMM) applying the 'lme'-function in the R library 'nlme' (Pinheiro *et al.* 2016) were used to test for plant diversity effects on DOM concentrations (see Appendix 2 for the full analysis model). Starting from a constant null model, with plot as random intercept and year as random slope, the null model was extended stepwise. Thereby, the random-effects term "plot" provided an error term for all fixed-effects terms that did not vary within plots among years and the random-effects term "time" provided an error for fixed-effects time contrasts such as precipitation. We fitted block as fixed effects as their number of three is too low to reliably estimate a variance component and because they do not fulfil the requirement of a random sample with normally distributed effects since they are systematically arranged in a linear sequence (Schmid *et al.* 2017). The fitting sequence of fixed terms followed the *a-priori* hypotheses of the biodiversity experiment starting with block followed by PSR (log-linear term); by plant functional group richness (linear term); and in alternative models the presence of all individual plant functional groups, as they are not independent of each other. Furthermore, sampling depth of the soil water (20 cm, 30 cm); the interactions between "depth" and plant diversity variables; season; the interactions between "season" and plant diversity variables; and the interaction between "depth" and "season" were tested. Plant functional group richness and the presence of grasses were never significant and were therefore removed from all analysis models. The response variable DOM concentration was log-transformed in order to obtain a normally distributed error structure and to stabilise variances. The maximum likelihood method was used and likelihood-ratio tests (*L*-Ratios) were applied to assess the statistical significance of stepwise model improvement. All variables were scaled to a range between 0 and 1 (Legendre & Legendre 1998) to improve model convergence. To test plant community effects on molecular DOM properties (m/z, H/C, and O/C), similar LMM were used as

described above, but only with plot as random effect as DOM properties were measured once in a single block.

### ***Identifying mechanisms underlying plant diversity effects on DOM***

Using the R library 'piecewiseSEM' (Lefcheck 2016), confirmatory path analyses were applied to test the causal relationships between plant diversity and DOM concentrations in different seasons. To identify the mechanisms underlying the plant diversity effects on DOM concentrations, we set up an initial path model (Appendix 3). This includes root biomass, microbial respiration, soil organic carbon contents at different soil depths, silt content of soil as a proxy for soil texture, and sampled water volumes as proxy for soil water percolation (Fischer *et al.* 2019) as possible mediators. The underlying drivers of the plant diversity effect were tested individually for each season based on the data gathered in 2011 and 2014 (years in which data were available for all considered DOM drivers). To account for the spatial (two sampling depths) and temporal (two years) dependence of measurements within each structural equation model, plot identity was fitted as random factor. In addition to the calendar seasons, the path analyses were conducted based on meteorological seasons classified as follows: winter is from December to February, spring from March to May, summer from June to August, and autumn from September to November.

## **Results**

### **Impact of plant diversity on DOM concentrations**

During our observation period from 2011-2016, the average DOM concentration was  $9.6 \text{ mg L}^{-1}$  ( $\pm 4.4$  SD,  $N = 9563$  of all fortnightly samples). However, DOM concentrations varied strongly with season (Fig 1a,b). In winter, the DOM concentration was lowest ( $7.7 \pm 3.5 \text{ mg L}^{-1}$ ), while the concentrations increased towards spring ( $8.6 \pm 4.4 \text{ mg L}^{-1}$ ), and reached their peak in summer ( $13.1 \pm 3.6 \text{ mg L}^{-1}$ ). The concentrations decreased again towards autumn ( $11.6 \pm 2.7 \text{ mg L}^{-1}$ ). Thus, the mean DOM concentrations in summer exceeded those in winter by almost 70%.

Besides the strong seasonal differences, DOM concentrations were significantly impacted by PSR and functional group composition (Appendix 4). DOM concentrations increased with PSR and in the presence of small herbs and legumes (Fig 1b,c). Among these plant community characteristics, PSR was

the most significant (PSR:  $L$ -Ratio = 13.82;  $P$ -value < 0.001, small herbs:  $L$  = 7.57;  $P$  = 0.006, legumes:  $L$  = 5.94;  $P$  = 0.015); DOM concentrations increased by about 23% in plots with a sown diversity of 60 plant species ( $11.3 \pm 4.0$  mg L<sup>-1</sup>) compared with monocultures ( $9.2 \pm 3.7$  mg L<sup>-1</sup>). These effects were consistent across all seasons and at both sampling depths (Fig. 1b; Appendix 4). The presence of tall herbs had no significant consistent effect on DOM concentrations. Instead, the presence of tall herbs affected DOM concentration to varying extents at different sampling depths, as indicated by the significant interaction term “tall herbs × depth” ( $L$  = 7.14;  $P$  = 0.008). At 30 cm sampling depth, DOM concentrations were higher in plots with tall herbs ( $10.5 \pm 4.6$  mg L<sup>-1</sup>) than in plots without ( $9.5 \pm 4.4$  mg L<sup>-1</sup>), while the concentrations did not differ at a depth of 20 cm (Fig 1c).

### **Plant diversity effects on molecular DOM properties**

PSR decreased the average molecular size ( $m/z$ ) of DOM ( $L$  = 5.94;  $P$  = 0.015), its average state of oxygenation (oxygen-to-carbon ratio:  $L$  = 5.89;  $P$  = 0.015), and its state of degradation assessed by  $I_{deg}$  ( $L$  = 4.33;  $P$  = 0.037). This means that smaller DOM molecules were found with increasing PSR, holding a lower oxygen-to-carbon ratio and being less degraded (Fig 2). The hydrogen-to-carbon ratio was not affected by PSR. Molecular DOM properties were not significantly affected by the presence of any type of functional group. Moreover,  $m/z$ , H/C and  $I_{deg}$  increased with soil depth ( $m/z$ :  $L$  = 7.73;  $P$  = 0.005, H/C:  $L$  = 24.99;  $P$  < 0.001,  $I_{deg}$ :  $L$  = 9.25;  $P$  = 0.002, Appendix 5), while the interaction term PSR x depth was never significant for any of the metrics of molecular DOM. This indicated that the strength of the PSR effect was the same at both depths and therefore smaller, less degraded, low O/C ratio DOM molecules can be found at both soil depths when there is a high PSR.

### **Mediators of the plant diversity effects on DOM concentrations during the course of the year**

Structural equation models were compiled on the basis of both calendar and meteorological seasons (see Methods). Only the statistical values for the calendar seasons are given here as the results are very similar for both seasonal classifications. The detailed modelling results for both classifications can be found in Appendices 6, 7 and 8.

Structural equation models indicated that the positive impact of PSR on DOM concentration was mainly mediated by soil microbial activity and, in specific seasons, by SOM in the top 5 cm of soil and root standing biomass (Fig 3a). However, the effect of these underlying drivers changed over the course of the year (Fig 3b). In summer and autumn, microbial activity was positively related to DOM concentrations, this became negative in winter and spring. The effect of SOM showed opposing patterns, namely it was

not related to DOM concentration in summer and marginally negative in autumn ( $P = 0.055$ ), before switching to a positive relationship in winter ( $P < 0.001$ ). Root standing biomass was only a weak predictor of DOM, showing a significant negative relationship with DOM concentrations in autumn ( $P = 0.043$ ). However, the direct paths of plant diversity and presence of small herbs on DOM concentration in specific seasons show that their effects were not always completely mediated by the fitted environmental variables.

Among environmental drivers of DOM concentrations, independent of plant diversity, soil silt content was most important; increasing silt content decreased DOM concentrations (Fig 3a). In addition to this strong, direct negative effect on DOM, the silt content indirectly impacted DOM positively due to its high SOM content. Furthermore, DOM concentrations were significantly influenced by the volume of soil water collected in each season. As for the other drivers, the effect of soil water changed from positive in the first half of the year to negative in the second half of the year. The negative effect of soil water volume on DOM concentration in the second half of the year indicates a dilution effect. In contrast, the positive effect in the first half of the year was strongly driven by SOM and, notably, was accompanied by a strong negative correlation between the volume of sampled soil water and soil microbial activity.

## Discussion

Based on a long-term grassland biodiversity experiment, our study demonstrates a strong plant diversity effect on DOM concentrations across all seasons of the years 2011-2016 and reveals for the first time a plant diversity effect on the molecular properties of DOM. DOM concentrations increased by about 23% from monocultures to highly diverse plant communities (60 species). However, the seasonal variations of DOM concentrations exceeded the plant diversity effects by far and also influenced plant diversity effects on DOM concentration.

### Seasonal variations in DOM concentrations

The seasonal patterns of DOM concentrations in our study support our hypothesis and are in line with the general observations that DOM concentrations in soil solution are higher in summer than in winter (Kalbitz *et al.* 2000), but see Don and Schulze (2008) for inconsistent findings. A peak in late summer has been reported frequently and is called the “rewetting peak”, as it can be observed after dry periods when the soil gets humid again (Kalbitz *et al.* 2000). Our study is in line with these observations,

with the highest DOM concentrations found in August (Appendix 9). Previous studies suggested that over the dry period either microbial products (Don & Schulze 2008) or plant-derived compounds (Karlowsky *et al.* 2018) accumulate in the dry soil. After rewetting of the soil, these accumulated compounds get leached and contribute to the high DOM concentrations. However, the DOM concentrations peak after such rewetting seems to depend on the soil type and its clay content, as Don and Schulze (2008) did not find a rewetting peak in a Vertisol that contains up to 70% clay. At the Jena Experiment site, the soil clay content was much lower with an average of 21%. Besides this peak in DOM concentration, higher DOM concentrations were generally observed from July to November (Appendix 9) compared to the period from December to May. This can be most likely attributed to higher biological activity, resulting in higher plant productivity and microbial degradation (Pietikäinen, Pettersson & Bååth 2005; Kadereit *et al.* 2014).

### **Plant diversity effects on DOM concentrations and molecular properties of DOM**

Regardless of the season, DOM concentrations increased with higher plant species richness and in the presence of legumes and small herbs. This consistency was unexpected and does not fully support our hypothesis of seasonality on the strength of the plant diversity effect as plant and microbial drivers of DOM have previously been reported to be strongly affected by seasonal changes in temperature, light and water availability (Pietikäinen, Pettersson & Bååth 2005; Kadereit *et al.* 2014). Thus, the positive effects of plant diversity and composition on DOM concentrations indicate higher biological activity with increased plant diversity. Plant diversity and composition effects on DOM concentrations were usually mediated via soil-related variables, such as the SOM content or the soil microbial activity. These variables were in turn, strongly influenced by plant diversity (please see our discussion on mediators of plant diversity and composition). However, in certain seasons the plant diversity effects were not completely conveyed by these mediators. The direct plant effects on DOM concentrations only occurred in the second half of the annual cycle and could be related to the rewetting peak indicating accumulation of plant-derived compounds in soil during dry summer periods as supposed by Karlowsky *et al.* (2018). Thus, with higher plant species richness and in the presence of small herbs, more plant-derived compounds are likely to accumulate during dry periods and then cause an increase in DOM concentrations in the following months.

Furthermore, the different effects of tall herbs at different soil depths suggests that rooting depth is an important root trait impacting DOM concentrations. The fact that the presence of tall herbs increased DOM concentrations at 30 cm soil depth but not above 20 cm indicates that deep-rooting plants enhance plant carbon allocation to deeper soil layers. Thereby, microbial activity can be enhanced with

potential consequences for decomposition processes in deeper soil layers. However, the presence of tall herbs or its interaction with depth was not confirmed by the path model, and there was no effect of tall herbs on molecular properties of DOM at any depth (Appendix 3). This indicates that the microbial decomposition is not restricted to plant material, which is further supported by a decrease in the mean molecular DOM size (Roth *et al.* 2019), but also to the decomposition of stored SOM. However, as the molecular properties of DOM were only measured on a reduced data set (see our discussion below), future studies are needed to further investigate this relationship.

Beside the effect of plant diversity on DOM concentrations, our study demonstrates an impact of plant diversity on molecular properties of DOM, supporting our hypothesis. The molecular differences in DOM indicate a plant diversity-mediated shift in the plant-microbial interplay of carbon production and decomposition. The decrease in mean molecular weight of DOM molecules with plant diversity is caused by a higher abundance of small molecules that are likely to be microorganism-generated during the very early decomposition of plant-derived carbon (Roth *et al.* 2019). This is in line with previous findings that plant diversity impacts low molecular weight compounds in soil (El Moujahid *et al.* 2017). With increasing soil depth, the mean molecular weight of DOM increases due to preferential consumption of small DOM molecules and microbial production of larger molecules (Roth *et al.* 2019). Thus, molecules of microbial origin become more dominant in DOM with increasing soil depth (Kaiser, Guggenberger & Haumaier 2004; Steinbeiss, Temperton & Gleixner 2008). Moreover, the higher O/C ratio that accompanies higher plant diversity shows that with higher plant diversity the molecules have a higher potential to be oxidized. In contrast, the H/C ratio, indicating the hydrogen saturation of carbon, was not related to plant diversity and composition but was strongly influenced by depth. This change in carbon saturation with soil depth (Roth *et al.* 2015) indicates independent mechanisms underlying the plant species richness induced changes in molecular DOM. However, with higher plant diversity, less degraded and more easily decomposable small compounds were found in deeper soil layers in the present study, indicating that the products of the early decomposition of plant inputs exceed the microbial consumption and reach deeper soil layers. Thus, with higher plant diversity, fresher and more reactive molecules are transported into deeper soil layers, which may foster a microbial life that initiates soil development, soil biodiversity, and soil fertility (Nielsen, Wall & Six 2015; Klopff *et al.* 2017). This molecular DOM transformation is not only restricted to the top 30 cm of the soil presented in our study, but is a more general phenomenon observed along the soil profile (Roth *et al.* 2019).

In contrast to the clear difference in the mean molecular weight of DOM, there was no difference in DOM concentration between sampling depths in our study. This indicates, firstly, that the legacy of

former land use as arable land with its homogenised plough horizon is still visible in the DOM concentrations and, secondly, that microbial processes cannot be fully understood without investigating molecular DOM properties. However, the molecular DOM analyses were limited to one time point and only 20 plots. For DOM from forest soils, it was reported that its molecular composition correlates with DOM concentrations and season (Roth *et al.* 2015). This indicates that there is a constant input and recycling of organic matter. However, we suspect that plant inputs as well as the microbial consumption and production are constantly driven by plant diversity, so the effect of plant diversity on the molecular properties of DOM is likely to persist irrespective of the season. Moreover, the block from which the samples for molecular DOM analyses were derived was located between a block with higher silt and a block with higher sand content, i.e. it represents “average soil conditions” of the experimental site. Nevertheless, we assume that the general plant diversity effect on molecular DOM metrics is not affected by these differences in soil conditions between blocks. The DOM transformation during its passage through soil in the Jena Experiment was confirmed at a different location with very sandy and acidic soil (Roth *et al.* 2019). This suggests a consistent influence of the DOM drivers, such as plant diversity, regardless of soil type and texture, although the strength of the drivers may be enhanced or attenuated. Moreover, Roth *et al.* (2019) showed that the explained variances in molecular DOM through plants and soil are independent of each other. However, investigating the molecular DOM on a larger set of plots and throughout the year, together with more detailed information on soil microbial community composition, is likely to provide more detailed insights and greater mechanistic understanding of how plant diversity affects belowground processes.

### **Mediators of the plant diversity and composition effects**

Structural equation modelling suggested that plant diversity effects on DOM concentration were mainly driven by increased microbial activity and SOM content in the topsoil. However, the effect of microbial activity and SOM content on DOM concentrations differed between seasons, indicating that the consistent plant diversity effect is mediated by different drivers in different seasons. Soil microorganisms have been found to act as both sinks and sources of DOM (Kalbitz *et al.* 2000; Neff & Asner 2001). In our study, microbial DOM consumption exceeded microbial DOM production in winter and spring, as indicated by the negative relationship between soil microbial activity and DOM concentrations. The positive relation between microbial activity and DOM concentrations in summer and autumn indicate that the microbial activity changed its impact on DOM during the year from consumption to production of DOM.

The relationship between DOM concentrations in soil water and SOM contents in the soil, which are significantly higher in more diverse plant communities (Lange *et al.* 2019), also changed during the year. In contrast to soil microbial activity, the relationship was positive in winter and changed during the year to negative in autumn. These patterns indicate that in plant communities with high diversity, SOM is mostly a source of DOM in winter, but later in autumn DOM is consumed with higher SOM contents. However, these relationships were weaker than those of DOM with the soil microbial community and they likely depend on soil microbial activity. In winter, when the microbial community is less active and less abundant (Habekost *et al.* 2008), SOM was positively related to DOM concentration, indicating that SOM leaching contributes to higher DOM concentrations. The tendency towards a negative relationship between SOM and DOM concentrations in autumn is likely to be related to increased consumption of the SOM leachates by the more abundant soil microbial community in highly diverse plant communities. In addition, the microbial community contributes to higher DOM concentrations by decomposition of dead plant residues in the second half of the growing season (Kuzyakov 2002).

Root standing biomass only mediated the plant diversity effects on DOM concentrations in autumn. The negative impact of root standing biomass on DOM concentration was counter-intuitive at first glance. Although the root standing biomass was shown to increase with plant diversity (Mueller *et al.* 2013; Ravenek *et al.* 2014) roots from plant communities with high diversity had lower decomposition rates (Chen *et al.* 2017) and longer lifespans (Solly *et al.* 2013). Moreover, Chen *et al.* (2017) reported that root decomposition rates increase in the presence of legumes and small herbs due to an increase in substrate quality, i.e. a decrease in root C/N ratio. This likely further explains the positive effects of legumes and small herbs on DOM concentrations, as in their presence root decomposition is accelerated and more decomposition products are found in the soil water.

We are aware of the fact that we relate data of different temporal resolutions, and that annual measurements of predictors can partly be too coarse to fully explain the variability of DOM concentrations in different seasons of the year. However, most predictors do not change over the course of the year, including soil properties (silt and SOM content) and experimental design variables (sampling depth, plant species richness). In contrast, soil microbial activity and root standing biomass are likely to vary between seasons. Although it was shown in the Jena Experiment that the soil microbial community was more abundant in autumn than in spring, the plant diversity effect was consistently positive in both seasons (Habekost *et al.* 2008). Root standing biomass is likely to be positively related to plant species richness throughout the growing seasons. Thus, these annual measurements may miss relevant variation between seasons as indicated by the direct paths of the experimental design variables in the structural

equation models (basically reflecting unexplained variance), but they still turned out to be powerful proxies as mediators for the plant diversity effect over the course of the year. Therefore, our study gives first insights into the shifts in mediators of the plant diversity effects between seasons indicating that ecosystem functioning varies seasonally. Future studies should link temporally highly-resolved measurements of predictors such as root production and decomposition as well as soil microbial community composition to DOM concentrations to further elucidate the drivers of DOM variability during the seasons.

### **Environmental drivers of DOM concentrations independent of plant diversity**

The negative effect of the silt content points to sorption processes that demobilise DOM and thereby decrease DOM concentrations (Kaiser & Guggenberger 2000; Kalbitz *et al.* 2005). Higher silt content in soil is often accompanied by a higher free sorption capacity that decreases DOM concentrations. Soil clay content, which is considered to control sorption and adsorption processes, is relatively constant in the Fluvisol of the Jena Experiment. In contrast, there is a strong sand-silt-gradient at the field site (Roscher *et al.* 2004). The negative impact of silt on DOM concentration is likely to be driven by the fine silt portion, which has been demonstrated to increase the sorption capacities of soils (Schleuss *et al.* 2014). These sorption processes together with microbial mineralisation of DOM cause a strong decline in DOM concentrations with soil depth (Kaiser & Kalbitz 2012). In our study, DOM concentrations did not differ between the soil depths we sampled, nor did the plant diversity effect on DOM concentration differ between sampling depths. This likely resulted from the small spatial distance of only 10 cm between the two sampling depths. In addition, the former land use caused a soil homogenisation in the upper 30 cm due to ploughing. However, the study of Roth *et al.* (2019), which took place in the same experiment, found a significant decrease in DOM concentrations towards 60 cm soil depth.

The changing effects of the sampled soil water volumes on DOM concentrations between seasons could reflect seasonally different interactions among the microbial community, SOM, and soil water. In summer and autumn, when the DOM production is highest (Kalbitz *et al.* 2000; Don & Schulze 2008), the negative effect of soil water on DOM concentrations indicates a dilution effect. In contrast, the positive effect of the sampled volumes of soil water on DOM in winter and spring indicates a faster vertical transport of soil water and its DOM to deeper soil layers, which is driven by higher SOM in the topsoil (Fischer *et al.* 2015; Fischer *et al.* 2019; Lange *et al.* 2019). This suggests on the one hand that in winter and spring water transports more dissolved components of SOM to deeper layers, and on the other hand

that this occurred in locations with substantial storage of SOM. Furthermore, the negative correlation between microbial activity and the sampled volume of soil water indicates that less microbial consumption and decomposition of DOM can take place when the soil water percolates faster through the soil.

### **Conclusions and implications**

This study demonstrates that DOM may be an appropriate proxy to investigate ecosystem functions and functioning. DOM integrates the highly complex and interwoven processes of ecosystems, and reflects them in the concentration and molecular composition of DOM. Plant diversity affects a wide range of DOM drivers and their interactions (Lange *et al.* 2019), and thus DOM itself as an important mediator of above-belowground coupling. DOM plays a crucial role in a multitude of processes, such as cycling and distributing of nutrients and carbon that are central to ecosystem functioning (Bolan *et al.* 2011; Jansen, Kalbitz & McDowell 2014). With increasing plant diversity, soil water with elevated DOM concentrations, which has been less processed by soil microorganisms, reaches deeper soil layers. Thus, more of the less processed DOM can be utilised by the subsoil microbial community (Bolan *et al.* 2011). This less processed DOM might then foster a more fertile soil, that enables, for instance, higher rates of carbon storage (Fornara & Tilman 2008; Lange *et al.* 2015) since DOM is, along with deep rooting plants, the most important carbon source on which the formation of subsoil carbon is based (Rumpel & Koegel-Knabner 2011). Thus, plant diversity stimulates above-belowground interactions using DOM to “activate” subsoils, which might in turn increase soil organic matter storage and soil fertility.

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### **Data Availability Statement**

Data on DOM concentration and soil organic carbon contents are available at <https://dx.doi.org/10.17617/3.52>. Data on molecular DOM properties are available at <https://doi.org/10.17617/3.28>; soil texture data are available at <https://doi.org/10.1594/PANGAEA.885439>; microbial respiration data are available at <https://doi.pangaea.de/10.1594/PANGAEA.854694>; root standing biomass from 2011 at <https://doi.org/10.1594/PANGAEA.880330>; and from 2014 at <https://doi.pangaea.de/10.1594/PANGAEA.880324>.

### **Authors' Contributions**

M.L. and G.G. conceived and designed the experiment; V-N.R. and T.D. provided data on molecular DOM. N.E., O.G.M., S.S. and T.S. provided data on microbial activity; L.M., N.J.O, J.R. and A.W. provided data for root standing biomass; M.L. and C.R. analysed the data; and M.L. lead led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. The authors have no conflict of interest to declare.

## Figure captions

**Fig 1:** DOM concentrations [ $\text{mg L}^{-1}$ ] (a) averaged per fortnightly sampling date at the field site (mean  $\pm$  standard deviation) from 2009 to 2016, (b) as function of plant species richness (PSR) in each season, and (c) as affected by the presence of legumes, small herbs and tall herbs at different soil depths. Given are the means and the standard errors of mean, the plot number is  $N = 62$ . Asterisks indicate a significant ( $P > 0.05$ ) effect. Please refer to Appendix S4 for the detailed statistical results of the full model. Note that to illustrate the seasonality of DOM concentrations, the time series (a) covers the time period from the end of 2008 to the end of 2016, but (b) and (c) and all statistical analyses are based on data from 2011 to 2016.

**Fig 2:** Plant species richness effect (PSR, log scale) on weighted means per plot at different sampling depth of (a) the molecular size of the DOM, given as mass-to-charge ratio ( $m/z$ ), (b) the oxygen-to-carbon ratio (O/C) of DOM molecules, and (c) an index of degradation ( $I_{deg}$ ). Soil water was sampled in May 2014 on 20 plots at depths of 20 cm and 30 cm. The likelihood-ratios ( $L$ ) with the respective  $P$ -values ( $P$ ) are given to indicate the PSR and the depth effect on each metric of molecular DOM. For detailed statistics, please see Appendix 5.

**Fig 3:** Structural equation models to identify the underlying drivers of the positive effects of plant species richness (PSR) and the presence of legumes and small herbs on DOM concentrations as well as root standing biomass (roots), soil organic matter (SOM) at sampling depth (plate) or at topsoil, microbial activity, and sampled soil water volume (Vol) as potential mediators. Shown are (a) specific models for each season (winter = Quarter 1 (Q1), spring = Q2, summer = Q3, autumn = Q4). Solid arrow lines represent positive path and dashed arrow lines represent negative path coefficients. Arrow line widths indicate the size of significant ( $P < 0.05$ ) standardised path coefficients. Numbers in brackets represent the conditional  $R$ -squared ( $R^2_c$ ) of the response variables (see Appendix 3 for marginal  $R$ -squared values and Appendix 4 for goodness-of-fit statistics). Bar graphs (b) show direct effects (standardized path coefficients) of experimental, plant and soil related variables on DOM concentrations, identified with the path models. Asterisks indicate a significant ( $P < 0.05$ ) and in brackets a marginally significant ( $P < 0.1$ ) impact of the displayed variable on DOM concentration.

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