Running Head: Plant structure drives soil functions

# The functional structure of plant communities drives soil functioning via changes in soil abiotic properties

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## Abstract

While biodiversity is expected to enhance multiple ecosystem functions (EFs), the different roles of multiple biodiversity dimensions remain difficult to disentangle without carefully designed experiments. We sowed plant communities with independent levels of functional (FD) and phylogenetic diversities (PD), combined with different levels of fertilization, to investigate their direct and indirect roles on multiple EFs, including plant-related EFs (plant biomass productivity, litter decomposability), soil fertility (organic carbon and nutrient pool variables), soil microbial activity (respiration and nutrient cycling), and an overall multifunctionality. We expected an increase in most EFs in communities with higher values of FD and/or PD via complementarity effects, but also the dominant plant types (using community weighted mean, CWM, independent of FD and PD) via selection effects on several EFs. The results showed strong direct effects of different dimensions of plant functional structure parameters on plant-related EFs, through either CWM or FD, with weak effects of PD. Fertilization had significant effects on one soil microbial activity and indirect effects on the other variables via changes in soil abiotic properties. Dominant plant types and FD showed only indirect effects on soil microbial activity, through litter decomposition and soil abiotic properties, highlighting the importance of cascading effects. This study shows the relevance of complementary dimensions of biodiversity for assessing both direct and cascading effects on multiple EFs.

Keywords: biodiversity effect, ecosystem functioning, functional diversity, litter decomposition, multifunctionality, phylogenetic diversity, plant–soil interaction, soil abiotic properties.

## 1. Introduction

Despite multiple studies on the effects of biodiversity on ecosystem functioning (Naeem et al. 1994, Tilman and Downing 1994), such a relationship is still unclear due to a high degree of complexity in assessing multiple biodiversity effects. On the one hand, biodiversity can be characterized by different, often non-independent dimensions, e.g. taxonomical, functional and phylogenetic (de Bello et al. 2017), where each can play different roles (Cadotte 2017). On the other hand, the fact that many ecosystem functions (EFs) are also non-independent with respect to each other increases the complexity of the relationship between EFs and biodiversity (Zavaleta et al. 2010, Lavorel and Grigulis 2012, Meyer et al. 2018, van der Plas et al. 2019). Finally, the effect of a given trophic level on certain EFs can be mediated, through cascade effects, by other trophic levels or other ecosystem properties (Valencia et al. 2018). Thus, to improve our understanding of the complex biodiversity–ecosystem functioning (BEF) relationships it is necessary separate the effects of biodiversity dimensions across multiple types of EF.

Among the different dimensions of biodiversity, awareness is increasing that the distribution of functional trait values in a community, rather than taxonomical diversity (usually species richness), chiefly controls EFs (Hooper et al. 2005, Díaz et al. 2007, Cernansky 2017, Cadotte 2017). Recently, the effect of plant species richness on EFs was shown to be mediated by different parameters of the community functional structure (Eisenhauer et al. 2018). At the community level, community weighted mean (CWM) and functional diversity (FD; Díaz et al. 2007) are likely to be among the main parameters of the trait distribution affecting EFs (Valencia et al. 2018). CWM is expected to mainly reflect the selection effect driven by traits of dominant species, while FD reflects the complementarity effect, or non-additive effects in general (Dias et al. 2013, Cadotte 2017). Since available and measured trait information might only reflect a subset of functionally relevant traits, researchers have further considered phylogenetic relatedness between species as a proxy (or complement) of trait differences, under the assumption that more closely related species are more likely to have similar trait values (Cadotte et al. 2013). If closely related species are similar in their traits, then FD and PD (phylogenetic diversity) should be related at least to some degree. Then, the independence of PD from FD depends on the identity of traits used to compute FD and the level of conservatism of those traits in the phylogeny (Webb et al. 2002, Flynn et al. 2011, de Bello et al. 2017). While many biodiversity experiments have aimed to evaluate the role of species richness on ecosystem functioning (Tilman and Downing 1994, Roscher et al. 2004, Fargione and Tilman 2005), we know much less about the independent and combined role of functional and phylogenetic structure (but see Pichon et al. 2020). Therefore, to test the relative roles of different dimensions of biodiversity on EFs, manipulative designs that avoids the non-independence of potential predictors (e.g. FD and PD) would provide a step forward in the understanding of the complex nature of BEF relationships.

Another open issue in understanding BEF relationships is that given communities provide a variety of EFs, often interrelated between them. However BEFs have been primarily studied through individual functions such as biomass productivity (Hector et al. 2002), resistance to weed invasion (Fargione and Tilman 2005) or nutrient loss (Scherer-Lorenzen et al. 2003). Over the past decade, quantitative tests for evaluating multifunctionality, i.e. the provision of multiple functions simultaneously (Zavaleta et al. 2010), have been developed (see Byrnes et al. 2014 for a review). EFs are often not entirely independent from each other but rather covary in bundles of related functions (Lamarque et al. 2014). In fact, trade-offs among different EFs (Meyer et al. 2018) may cause the absence of biodiversity effects on multifunctionality, highlighting the interest of evaluating each individual function both separately and in combination. This can be achieved by investigating direct and indirect effects between the functions and their drivers. For example, plant communities, as primary producers, are a strong driver of local environmental conditions, affecting directly or indirectly (so-called cascading effects) the EFs controlled by other trophic levels (Lavorel et al. 2013). Previous experimental work showed that the plant-species-richness effect on soil multifunctionality was mediated by changes in plant community functional structure and soil microbial communities (Valencia et al. 2018). Effects on soil ecosystem properties can occur via litter decomposability, which is strongly related to species trait syndromes (Garnier et al. 2004, Pérez-Harguindeguy et al. 2013, Pichon et al. 2020) and particularly to species positions on the acquisitive–conservative strategy continuum (Wright et al. 2004, Díaz et al. 2016). Differences in litter decomposability trigger different rates in soil processes such as mineralization of organic matter and C sequestration (Hättenschwiler et al. 2005, Hobbie 2015). Plant traits related to nutrient content and decomposability of leaves, shoots and roots, as well as root architecture or nutrient uptake efficiency, can thus influence soil microbial community composition and functions by changing resource availability and local microclimatic conditions (de Vries et al. 2012, Moreau et al. 2015, Colin et al. 2019, De Long et al. 2019). Given the strong links between plant communities and ecosystem functioning, we argue that assessments of the role of plant community functional structure on EFs would improve if they consider possible cascading effects via changes in litter decomposition and /or in abiotic properties (Figure 1 summarizing possible direct and indirect drivers over multiple EFs).

In the present study, we used a grassland BEF experiment designed with fixed species richness and independent levels of both FD and PD to investigate the role of ecological differences between species on multiple EFs. We used two levels of fertilization, which is a key driver of plant communities and EFs (Bobbink et al. 2010) and may modulate the relationship between plant traits and soil. We ask the following questions: i) what are the relative effects of different parameters of plant community functional and phylogenetic structure and fertilization levels on individual ecosystem functions/properties and multifunctionality? ii) to which extent these effects are directly affecting soil microbial activity (respiration and enzymatic activities related to carbon, nitrogen and phosphorous cycling) or are indirectly mediated by plant-related EFs (plant aboveground biomass production, litter decomposability), soil abiotic conditions (acidity, salinity and moisture) and soil fertility (organic carbon and nutrient pools)? We hypothesized that i) higher EF values are associated with higher values of FD and/or PD, i.e. higher ecological differentiation between species, due to niche complementarity and non-additive biodiversity effects; ii) the dominant plant types, i.e. as reflected by CWMs, is expected to drive selection effects on different EFs. For instance, communities dominated by species with conservative strategies are associated with lower litter decomposability and productivity (Wardle et al. 2004, Lavorel and Grigulis 2012); iii) functional and phylogenetic structure might explain the activity of decomposers either directly or indirectly via changes in plant-related EFs that affect soil abiotic conditions; and iv) fertilization affects soil microbial activity, since this treatment might directly impact several soil related EFs, but also indirectly via its effects on plant communities (Barnard et al. 2006, Sardans et al. 2008).

## 2. Material and Methods

### 2.1. Experimental site and design

A field experiment was established in 2015 on a mesic meadow in the Czech Republic at an elevation of 660 m (Vysočina region, 49.331N, 15.003E). The mean annual temperature is 6.7°C and average annual rainfall is 759 mm (data from Černovice meteorological station, 4 km from the site). The study site is an abandoned crop field used for organic farming, last cultivated in 2001 and ploughed in 2014 prior to the experiment.

We selected a species pool of 19 species that naturally occur in similar habitats. Prior to the experimental setup, five quantitative traits and three qualitative (categorical) traits related to the competitive ability and niche occupation of the species were obtained from the LEDA trait database (Kleyer et al. 2008) and BiolFlor (Kühn et al. 2004) and used to design the experiment. The quantitative traits were canopy height (m), seed mass (mg), specific leaf area (SLA, $mm^2/mg$), leaf dry matter content (LDMC, mg/g) and length of flowering period (month 1–12), and the categorical traits were lifespan (annual/perennial), growth form (erosulate/hemirosette/rosette) and nitrogen-fixing ability (present/absent).

The experimental design was completely randomized and was based on plant communities with a constant sown species richness (six species) but contrasting levels (high/low) of FD and PD resulting in four combinations. We did this by simulating all potential combinations of six species from the pool and estimating their values of FD and PD. As measures of FD and PD, we used the Rao diversity index (Rao 1982) based on the trait average differences (Gower distance, for FD) and evolutionary distance (for PD) between species. Out of the possible mixtures, 10 communities were randomly selected from each of the four combinations of high and low values of FD and PD. While we focus closely on disentangling FD and PD roles, we also made sure the resulting communities did not include any unimodal or linear relationship between FD, or PD and CWM. Such relationships would stem from the fact that communities with extreme CWM values are generally composed by species with similar trait values (i.e., most species have either high or low trait values), and hence the FD values of these communities will always be small (as discussed in Dias et al. 2013). To avoid this, we excluded potential communities with extreme CWM, FD and PD values, resulting in communities in the experiment having independent CWM values from both FD and PD (see Galland et al. [2019] for a more detailed description of community selection). In addition to the 40 communities with six species combinations, three monoculture replicates of the 19 species were sown (sowing densities to all species used in Appendix S1: Table S1). Finally, the entire setup was replicated on fertilized and unfertilized plots, resulting in a total of 196 plots. Two extra monoculture plots were sown: unfertilized *Lotus corniculatus* and fertilized *Plantago media*. On each fertilized plot, fertilization was applied as dried, composted cow manure (2.2 t/ha, 33 kg N/ha, 55 kg $P_2O_5$/ha, 33 kg $K_2O$/ha) every year at the beginning of the growing season (March).

In the spring and fall of 2015, all the communities were sown with seeds from a local commercial supplier (Planta Naturalis). The experiment was designed as a fully randomized factorial design, with three treatments: functional diversity (high/low), phylogenetic diversity (high/low) and fertilization (with/without). Each of the 196 plots was 1.5 × 1.5 m, with a buffer zone of 0.5 m between them to avoid a possible edge effect (more information in Galland et al. [2019]) .

## 2.2. Plant trait and diversity

We measured *in situ* traits after the experiment was established in order to obtain the functional trait information from both the general environmental conditions of our experimental field and intraspecific variability within our experiment. The functional traits were measured following standard protocols (Pérez-Harguindeguy et al. 2013). Height, SLA and LDMC were measured in June 2016 for each of the 19 species on two individuals (one leaf per individual) per plot where the species were sown (22 to 40 samples per species per fertility level). Leaf nutrient content (carbon [C], nitrogen [N], phosphorus [P], C:N and N:P ratios) was measured in September 2016 on five samples per species per fertilization level: one monoculture and one of each of the four diversity levels. Total C and N concentrations were measured by dry combustion (Nelson and Sommers 1996) using a CHNS Elemental Analyzer vario MICRO cube (Elementar Analysensysteme GmbH, Germany). Total P was obtained by flow injection analysis (FIA). All quantitative trait values (except ratio) were ln transformed. For the analyses, all functional structure indices were recomputed with *in situ* trait values and observed biomass (g), which was obtained from ln transformed (ln[x+1]) biomass measurement of July 2017 (see below).

The final functional dissimilarity between species values used in the analyses were computed using the Traits Probability Density (TPD) framework (Carmona et al. 2016). The TPD approach can accommodate intraspecific trait variability between species in a multivariate space. To compute TPD, we first selected the traits which had correlation coefficients between them below 0.7 (Appendix S1: Figure S1) in order to avoid specific traits having a large effect on the combined functional diversity. Note that r values over 0.7 are often associated with levels of collinearity that cause problems with parameter estimation in statistical modelling (Dormann et al. 2013). The final traits selected to describe the functional differences between species were height, SLA, LDMC, leaf P and C:N ratio. For each fertilization level, using individual species means and standard deviations for each trait together with the correlation structure between the traits, we simulated trait combinations for a population of 100 individuals for each species based on a multivariate normal distribution (function *mvnorm* in package MASS, Venables and Ripley 2002). Then a PCA was computed based on those five selected traits for the 19 populations, and the scores of individuals on the two first axes were used as "trait" values to compute the TPD functions of each species (variance explained: 68.3%, Figure 2A). The functional pairwise dissimilarity between species was calculated based on the TPD overlap between species (Carmona et al. 2019) and used to compute the Rao diversity index for functional diversity (FD). Moreover, we used PCA axis scores of species centroids and observed biomass (ln[x+1]) in each community to compute CWM indices. For each community, higher values of CWM-PCA1 represent communities dominated by taller species with higher LDMC and low leaf P. Higher values of CWM-PCA2 represent communities dominated by species with higher leaf C:N ratio and lower SLA. This axis separates a group of legumes from the other plants, as these have particularly low C:N and tend to cluster at the negative pole (Figure 2A).

Phylogenetic relationships between species were extracted from an ultrametric supertree of European plant species, 'Daphne' (Durka and Michalski 2012), which was pruned to obtain a subtree with our 19 species. The phylogenetic distances between species were computed based on branch length information included in the Daphne supertree. These distances were used together with observed ln transformed (ln[x+1]) biomass (see below) to compute the Rao phylogenetic diversity (PD) of each community. We largely achieved the desired independence between FD, PD, and CWM (see Pearson correlation among the variables in Appendix S1: Table S2), so that this is ideal for disentangling the effects of these facets on EFs.

### 2.3. Vegetation biomass, diversity effect and weed colonization resistance

In the first week of July 2017 (i.e. two years after sowing), at the peak of the vegetative season, the aboveground biomass of each plot was clipped (2 cm aboveground) in a 50 x 50 cm quadrat. The biomass was sorted into individual sown species, while potential colonizing species were pooled into another sample. The samples were dried at 70ºC for 48 h before weighing. The colonizing species biomass was used as a proxy of the sown community vulnerability to colonization. Note that the biomass of colonizing species was always limited (Galland et al. 2019) and its effect on EFs was then considered to be secondary with respect to the sown species.

The experimental design with biomass sampling of individual sown species in monocultures and mixtures allowed us to evaluate the net diversity effect and its partitioning into complementarity and selection effects as proposed by Loreau and Hector (2001). The net diversity effect is estimated by the difference between the observed and the expected community yield based on species performances in monoculture. Specifically, the expected yield is the weighted (by the initial relative abundance of species in the community) average yield from the monocultures of species that compose the community. In the present experiment, the initial sowing density aimed to approximate equal abundance between species by adjusting both sowing density (seed number) and sowing mass (seed mass) (see details in Galland et al. 2019), so the expected yield was equivalent to a non-weighted average yield. The partitioning of the net diversity effect into complementarity and selection effects is based on the formula of Loreau and Hector (2001).

### 2.4. Litter decomposability

We conducted a litterbag experiment to evaluate plant community decomposability, following a similar protocol to Cornelissen (1996) and Finerty et al. (2016). On each plot, we collected senescent leaves at the end of the 2016 growing season (from the end of September until the end of November) from all species present (sown and colonizing species). We filled three replicate litter bags with 3 g of dried material and kept the litterbags on a homogenized sand bed for 5 months (see Appendix S2 for methodological details on the decomposition experiment). Finally, for each plot we estimated the averaged complement of the ratio between the final weight and the initial weight (decomposition = 1 - [$\mathrm{Weight}_{\mathrm{FINAL}}/\mathrm{Weight}_{\mathrm{INITIAL}}$]) from the three replicate samples, and used it as an indicator of community litter decomposability.

### 2.5. Soil sampling and analysis

After the biomass sampling in July 2017, we collected one soil sample per plot, which was composed of three soil cores (3 cm in diameter and 10 cm in depth) evenly distributed in the central square meter of the plot to account for spatial heterogeneity while avoiding edge effects. The pooled samples were sieved on a 2 mm mesh and split into two: a 20 g sample was frozen at -20ºC while the rest was oven dried (60°C, 48 h). Dried samples were used to measure pH and electrical conductivity (EC, $\mu S\ cm^{-1}$). Frozen samples were stored, and subsequently thawed at 5°C to measure soil gravimetric humidity as the weight loss after oven drying (105°C) (GH, %). Total organic carbon (TOC, g $kg^{-1}$ dw), total nitrogen (TN, g $kg^{-1}$ dw), total potassium (K, g $kg^{-1}$ dw) and total phosphorus (P, g $kg^{-1}$ dw) were analyzed in ground samples following standard protocols as in Navarro-Cano et al. (2015). In the same root-free sieved samples, we measured microbial $CO_2$-C production during aerobic incubation in the dark (ca. 12 g soil, 60% water-holding capacity, 28°C, 28 days) using a 6700 Headspace $CO_2$ analyzer (Illinois Instruments). We fitted the curve of cumulative $CO_2$-C production over time to a sigmoidal equation with three parameters ($y = a/(1 + \exp -(\frac{x-x_0}{b}))$; $R^2 \geq 0.95$, in all cases) in SigmaPlot v10.0. We estimated the kinetic parameters of soil microbial respiration (a, maximum degree of $CO_2$-C production; b, slope of $CO_2$-C production) and used the slope as a proxy for microbial productivity. Enzymatic activities related to C (β-glucosidase, GA) and P (acid phosphatase, PA) cycling were quantified as the amount of p-nitrophenol (PNP) that 0.5 g of soil produced under controlled conditions (temperature 37°C, 1 h and pH 6) (Tabatabai and Bremner 1969, Eivazi and Tabatabai 1988). Enzymatic activity related to the N cycle (urease, UA), which catalyzes the conversion of urea into carbon dioxide and ammonia, was quantified colorimetrically as the $NH_4^+$ produced after incubating (37°C, 2 h) 1 g of soil in 4 ml borate buffer (pH 10) and 0.5 ml of 0.48% urea (Kandeler and Gerber 1988).

The soil abiotic properties are those parameters not directly linked to fertility but which affect soil microbial community composition and activity or access to organic substances: pH, EC and GH. The soil fertility variables were the pools of TOC and macronutrients (total N, P and K), which are basic resources for the majority of heterotrophic soil microorganisms. Finally, we used microbial respiration (the slope of the $CO_2$-C accumulation curve of microbial respiration, rslope) and enzymatic activities related to C (GA), P (PA) and N (UA) cycling as indicators of soil microbial activity (Navarro-Cano et al. 2015). A PCA combining these variables showed a direct correlation between soil fertility and soil moisture, with the decomposition of organic matter, and the hydrolysis of C and P (positive pole, Dim 1; Figure 2B). Similarly, EC was positively correlated with the previous variables as typically occurs under carbon and nutrient enriched conditions due to a higher amount of ions in the soil solution. Soil pH was inversely related to TOC, as expected based on the acidic character of organic matter (negative pole, Dim 1; Figure 2B).

### 2.6. Assessing multiple ecosystem functions/properties

The evaluation of multiple EFs simultaneously has been a central goal of methodological development concerning multifunctionality (see Byrnes et al. [2014] for a review of different methodologies). Two of the most common approaches are the averaging method (Hooper and Vitousek 1998, Maestre et al. 2012) and the multiple threshold method (Byrnes et al. 2014); both are considered in the present study. The averaged multifunctionality takes the mean of a set of standardized EF values per plot using Z-score transformation. High values of this index mean high values for the different EFs evaluated, providing an easily interpretable summary of the measurements. We used the averaging methodology to estimate two averaged multifunctionality indices. The first one (MultF. soil) is a soil multifunctionality index and focuses on soil functions (fertility, respiration and nutrient cycling). The second one (MultF. all) combines soil and plant functions (biomass of sown community, resistance to colonization, litter decomposability).

Additionally, a multiple threshold approach was used to compare the results among the indices, as this approach performs well even in the presence of trade-offs among EF variables (Byrnes et al. 2014). We plotted the slope of the relationship between a predictor and multifunctionality across a range of thresholds from 5 to 95%. In the curve obtained, when there is no overlap between the 95% confidence interval and the zero line for a given threshold, it means a significant relationship between the multifunctionality at that threshold and the predictor. Therefore, this indicates the percentage of functioning at which the changes in the predictor has influenced multifunctionality.

### 2.7. Statistical analysis

We first explored how the functional and phylogenetic structure of plant communities together with the fertilization treatment affected individual ecosystem functions/properties and averaged multifunctionality indices. We used linear models and predictor selection procedure based on the Akaike information criterion (AIC) to obtain the best model for each response variable. Fertilization, CWM-PCA1, CWM-PCA2, FD and PD were the predictors in these models.

In a second step, we included functional and phylogenetic structure parameters, plant biomass productivity, decomposability and fertilization as predictors before running the selection procedure. The aim of this step was to investigate if the plant EFs (biomass and decomposability) are better predictors of individual soil ecosystem functions/properties and multifunctionality than plant functional and phylogenetic structure. Also, we conducted a similar model selection including soil abiotic properties and soil fertility as additional potential predictors of individual soil EFs (soil microbial activity) and multifunctionality indices. Since soil nutrient pool variables were well correlated with each other (Figure 2B) we computed a fertility summary variable for the nutrient pool using the average multifunctionality method (i.e. MultF-NutPool is the mean of the four Z-transformed carbon and nutrient pool variables TOC, TN, P and K) to reduce the number of mediator variables. Note that we also tested whether the inclusion of the interactions between fertilization and the predictors considered improved the models described above (i.e. whether the explained variance increased). However, we removed such interactions since they did not explain much additional variation.

Furthermore, we tested i) the full model with fertilization and all functional and phylogenetic structure parameters as predictors, and ii) the best model selected for averaged multifunctionality with the multiple thresholds approach (Appendix S1: Figure S2). Since the results were consistent with the averaged multifunctionality method, we present the results of the multiple threshold analysis only in the appendix (Appendix S1: Figures S3 and S4).

To test the direct effects of fertilization and plant community functional and phylogenetic structure on soil microbial activity, and also the indirect effects on this activity mediated by decomposability and soil abiotic parameters, we conducted a confirmatory path analysis using the d-sep approach (Shipley 2013) and the piecewise SEM package (Lefcheck 2016). This approach has certain characteristics that differentiate it from standard structural equation models. For example, it allows for the inclusion of non-linear relationships among variables, non-normal data distributions and small sample sizes (Grace 2006, Shipley 2009). We selected the most appropriate predictors for each soil microbial activity variable (rslope, GA, PA, UA) using the selection procedure described above. For instance, biomass productivity of sown species reduced microbial respiration (Appendix S1: Figure S2). However, after considering the partial effect of soil nutrient pool (Appendix S1: Figure S5), the effect of biomass on microbial respiration ceased to be significant. Therefore, plant biomass productivity was removed from the piecewise SEM to simplify the model (i.e.

reducing the number of variables). The total effect of each predictor was calculated as the sum of direct and indirect effects on each soil microbial activity variable. Additionally, standardized path coefficients were used to measure the direct and indirect effects of fertilization, and community functional and phylogenetic structure (CWMs, FD, PD), plant community EFs (plant biomass and litter decomposability), and the summary variable for the nutrient pool (MultF-NutPool) on each soil microbial activity variable (Grace and Bollen 2005).

All analyses were conducted with R software version 3.3.2 (R Development Core Team 2018) using different R packages.

## 3. Results

### 3.1. Effects of plant functional structure on individual EFs

Biomass productivity was the EF best explained by plant community functional structure (Figure 3, $R^2 = 0.28$). Communities with greater values of CWM-PCA1 (higher plant height and LDMC), CWM-PCA2 (higher C:N and lower SLA) and FD showed greater sown community biomass (Figure 3). Furthermore, high values in both CWM-PCA1 and CWM-PCA2 values had a positive effect on the net biodiversity effect, i.e. a larger increase with respect to the productivity of monocultures. Selection and complementarity effects responded to different parameters of the community functional structure. The selection effect increased with fertility and CWM-PCA2 while the complementarity effect increased under the combined effect of FD and CWM-PCA1.

Communities with greater CWM-PCA2 values were more productive and more resistant to weed colonization. The litter decomposability of the communities was lower when sown communities had higher values of CWM-PCA2 and FD (Figure 3). Interestingly, the fertilization treatment had no impact on plant biomass productivity, although it had a significant positive effect on the selection effect (Figure 3).

Overall, functional structure was a better predictor of soil abiotic properties (pH, EC, GH) than soil EFs (Figure 3). In particular the pH of the soil decreased as CWM-PCA2 and FD increased (Figure 3 and Appendix S1: Figure S6). EC and GH tended to decrease with higher values of FD and CWM-PCA2, respectively, although those relationships were only marginally significant (Figure 3). The fertilization treatment had a positive effect on pH and EC.

The soil organic carbon and nutrient pool variables (TOC, TN, K and P) showed, in our models, no direct relationship with any plant community functional or phylogenetic structure variables. The variables related to microbial activity (rslope, GA, PA and UA) showed a weak but significant relationship with the functional structure of plant communities (Figure 3 and Appendix S1: Figure S7). In particular, soil respiration decreased with decreases of FD gradient, as phosphatase activity did along the CWM-PCA2 gradient. Fertilization had opposite effects on glucosidase and urease activities, which increased and decreased respectively. Neither functional/phylogenetic community structure nor fertilization treatment had direct effects on either multifunctionality index (MultF. all and MultF. soil). PD was not selected as a significant predictor for any of the response variables.

### 3.2. Cascading effect of plant community on soil EFs

The results from the path analysis (Figure 4) showed that the effects of plant community functional and phylogenetic structure on soil EFs are essentially mediated by decomposability and by soil abiotic variables. The results were consistent with the separate models that add plant community EFs (plant biomass and litter decomposability, Appendix S1: Figure S2) and soil abiotic parameters (Appendix S1: Figure S5) to the initial model.

The main driver of soil microbial activity was the pool of organic carbon and nutrients (MultF-NutPool), which acted via both direct and indirect effects mediated by the soil abiotic conditions (EC, pH and GH; Figure 4 and Appendix S1: Figure S8). None of the functional or phylogenetic structure parameters nor the fertilization treatment were significant drivers of organic carbon and nutrient pools (neither as an aggregated index, Figure 4; nor as individual variables, Figure 3 and Appendix S1: Figure S1)

Greater FD and CWM-PCA2 were associated with lower levels of litter decomposability and impacted some abiotic parameters of the soil (Figure 4). Also, the CWM-PCA2 gradient had a direct negative effect on PA. The effect of CWM-PCA1 on soil EFs was essentially mediated through its positive, albeit weak, effect on litter decomposability. High decomposability was related to low GH but had no direct effect on any of the soil EFs measured (Figure 4). However, the soil EFs were well predicted by the soil abiotic properties and, also, indirectly via decomposability, CWM-PCA2 and FD (Figure 4 and Appendix 1: Figure S8). PD remained a significant direct predictor of acid phosphatase activity after the inclusion of soil nutrient pool and other abiotic soil parameters (Figure 4 and Appendix S1: Figure S5).

## 4. Discussion

This study shows the importance of disentangling the potential effects of ecological differences between species in affecting – directly or indirectly – EFs. The results show the importance of plant functional structure in predicting biomass productivity- and decomposition-related EFs (biomass of sown community, community resistance to colonization by weeds and community decomposability), with little additional value from phylogenetic differences. In return, these changes in plant EFs, and especially in litter decomposability, can have a direct consequence on soil conditions and EFs. In contrast, the effects of plant functional and phylogenetic structure on soil ecosystem properties are limited. Finally, fertilization has direct and indirect effects on soil microbial activity, via modification of soil abiotic variables but not via litter decomposability nor the organic pool. Our results support the importance of the cascading effects of plant functional structure and fertilization on soil EFs, which are mediated through both shifts in litter decomposability and soil abiotic properties (Figure 4, Appendix S1: Figures S2 and S5).

### 4.1. Effects on individual EFs and multifunctionality

Theoretical and empirical studies suggest that different dimensions of biodiversity support different aspects of the BEF relationship. In particular, complementarity is expected to increase with increasing FD, and the selection effect is expected to be influenced more by CWMs (Cadotte 2017). Our results provide nuanced support for these hypotheses, with indeed selection effects driven by CWM-PCA2, reflecting adquisitive-conservative trade-offs, and complementarity increasing with greater FD. However, complementarity also depended from the dominant trait values (CWM-PCA1 reflecting size relate traits) and FD was not related to the net diversity effect. These latter results seems partially counter-intuitive although they are not isolated in the literature. For example, Mahaut et al. (2020) reported experimental results where the complementarity effect was essentially related to CWMs. In light of the relationship between niche differentiation and competitive ability, as described by Mayfield and Levine (2010), a potential mechanistic explanation as to why

CWM-PCA1 is associated with the complementarity effect on biomass production could be its relation to CWM-height (pearson R = 0.71, p-value < 0.001). Because average trait values are driven by the dominant species and the dominant species is usually the tallest species, the potential for vertical complementarity in the vegetation layers is greater when the dominant species of a community is taller. Hence, when the dominant species is tall, then the short subordinates can coexist under the condition that they tolerate partial shading (e.g. plots with *Achillea millefolium*, the tallest species in our study, showed more diversity in height among the other species). Overall, a tall dominant species can also include subordinates, but a short dominant simply does not leave physical space for vertical complementarity.

Litter decomposability was strongly and negatively related to CWM-PCA2 (Figure 3), with leaf related traits associated with it being interpreted as a proxy for litter quality of the community (high CWM-PCA2 values represent low quality). Litter quality is particularly associated with low values of C:N ratio in leaf chemical composition (Pérez-Harguindeguy et al. 2000, Pálková and Lepš 2008). C:N ratio is a trait well represented on the second axis of the trait PCA (Figure 2A) and CWM-C:N is strongly correlated with CWM-PCA2 (Pearson R = 0.85, p-value < 0.001). CWM-PCA2 is also negatively correlated with SLA, so, again, low litter decomposability is associated with low SLA. These results indicate that communities dominated by species with conservative strategies (i.e. lower SLA) are associated with lower litter decomposability (i.e. slower turnover) and lower productivity (Wardle et al. 2004, Lavorel and Grigulis 2012). No non-additive effects between plant species seems to be at play in our study system, as litter decomposition was negatively associated with FD. Those results are consistent with the recent findings of Finerty et al. (2016) and Pichon et al. (2020) showing that decomposability increased when community functional composition was dominated by species with an acquisitive trait syndrome. Pichon et al. (2020) also reported a positive, indirect effect of species richness on litter decomposition, but no statistically significant effect of functional diversity, while Finerty et al. (2016) found both positive and negative FD effects depending on CWM values.

Soil abiotic properties showed a direct response to the experimental treatment in our models. In particular, fertilization increased pH and EC (Figure 3), as typically occurs with manure amendments. We did not detect a significant increase in carbon or nutrient pools with fertilization. However, soil glucosidase activity increased and urease activity concurrently decreased as a result of supplying this fertilizer which is rich in organic nitrogen. Community functional structure also impacted soil abiotic properties and microbial activity in the soil (i.e. respiration and phosphatase activity). On the one hand, the relationships between community functional structure and soil abiotic properties could be related with root exudates, ions uptake, vegetation effects via changes in micro-climatic conditions or in organic matter. However, a new study is needed to explore the ecological mechanism of the statistical relationships between CWM and FD considered here and soil abiotic propierties. On the other hand, phosphatase activity responded positively to plant community phylogenetic diversity (Appendix 1: Figure S5), which is consistent with previous findings (Navarro-Cano et al. 2014) and can result from two mechanisms (Goberna et al. 2016): i) plant phylogenetic diversity can stimulate soil microbial phylogenetic diversity via niche differences, stimulating microbial activity via the complementarity effect; ii) plant phylogenetic diversity can reduce microbial phylogenetic diversity via increased fitness differences, stimulating microbial activity by selection of highly competitive clades. As for litter decomposition, CWM-PCA2 and FD had a negative effect on microbial activity variables and, as discussed below, the effect of community functional structure on soil properties is in fact mediated by litter decomposition.

We did not find any strong direct effect of community functional or phylogenetic structure, or fertilization treatment, on averaged or multi-thresholded multifunctionality indices. These results can be explained by the lack of synergy, or even trade-offs, either among individual EFs (Figure 2B and Appendix S1: Figure S9) or in response to plant community functional structure. Indeed plant-related EFs, biomass productivity and litter decomposability, all showed the same opposing responses to CWM-PCA2 and FD. Regarding soil functions, none of the four nutrient pool parameters showed a response to any treatment, but all four microbial activity measures responded to either fertilization treatment (GA, UA) or community functional structure (rslope, PA). This observation could be the result of different timelags in responses to treatments; while the microbial community can respond relatively quickly, changes in nutrient pools can take longer (Haynes and Naidu 1998). In summary, our results point towards the presence of a trade-off between the EFs linked to biomass productivity and the other variables, i.e. while the biomass of the sown communities and their resistance increase along CWM-PCA2 and FD increase, the soil becomes more acidic, and litter decomposability and soil microorganism respiration reduce (Figure 3). This lack of synergy or even trade-off among individual EF responses could explain the absence of significant responses from multifunctionality analysis, as suggested by Meyer et al. (2018). However, other potential explanations are that the effect may be mediated by other variables or that some of the EFs do not respond to biodiversity (Allan et al. 2013).

### 4.2. Cascading effect on soil EFs

CWM-PCA2 and FD were the variables most often selected in the models predicting individual ecosystem properties from the plant community functional structure. The soil EFs were best predicted by a combination of the direct effect of plant community functional structure and their indirect effect through litter decomposition and soil abiotic properties, highlighting the need for considering a combination of different parameters to improve predictions of EFs.

Microbial respiration was the soil EF for which the cascading effect of plant community functional structure was most evident. The most dominant plant species in communities are located in the upper right corner of the PCA in Figure 2A, and they are characterized by conservative traits (tall species with high LDMC [PCA1], low SLA and high C:N ratio [PCA2]). These characteristics mean that communities with high values of CWM-PCA1 and CWM-PCA2 build up large biomass with slowly decomposing material (Pérez-Harguindeguy et al. 2000). High biomass productivity and slow litter decomposition were associated with slight but significant acidification of the soil, which was, in turn, associated with slower respiration in the soil.

## 5. Conclusion

Our study highlights the richness of plant-soil interactions and the necessity to include different dimensions of plant biodiversity, particularly focusing on plant traits, in the study of multiple EFs. As expected, the amount of soil organic carbon and the nutrient pool were the main drivers of rates of microbial decomposition and nutrient cycling. Still, even just two years after manipulating the functional structure of the plant communities, we observed its impact on soil functions, particularly through the effect that plant traits exerted on soil abiotic properties. Given the experimental nature of our study, this provides evidence for cascading effects from primary producers to decomposers.

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## References

Allan, E., W. W. Weisser, M. Fischer, E. D. Schulze, A. Weigelt, C. Roscher, J. Baade, R. L. Barnard, H. Beßler, N. Buchmann, A. Ebeling, N. Eisenhauer, C. Engels, A. J. F. Fergus, G. Gleixner, M. Gubsch, S. Halle, A. M. Klein, I. Kertscher, A. Kuu, M. Lange, X. Le Roux, S. T. Meyer, V. D. Migunova, A. Milcu, P. A. Niklaus, Y. Oelmann, E. Pašalić, J. S. Petermann, F. Poly, T. Rottstock, A. C. W. Sabais, C. Scherber, M. Scherer-Lorenzen, S.

Scheu, S. Steinbeiss, G. Schwichtenberg, V. Temperton, T. Tscharntke, W. Voigt, W. Wilcke, C. Wirth, and B. Schmid. 2013. A comparison of the strength of biodiversity effects across multiple functions. Oecologia 173:223–237.

Le Bagousse-Pinguet, Y., S. Soliveres, N. Gross, R. Torices, M. Berdugo, and F. T. Maestre. 2019. Phylogenetic, functional, and taxonomic richness have both positive and negative effects on ecosystem multifunctionality. Proceedings of the National Academy of Sciences of the United States of America 116:8419–8424.

Barnard, R., X. Le Roux, B. A. Hungate, E. E. Cleland, J. C. Blankinship, L. Barthes, and P. W. Leadley. 2006. Several components of global change alter nitrifying and denitrifying activities in an annual grassland. Functional Ecology 20:557–564.

de Bello, F., S. Lavorel, S. Díaz, R. Harrington, J. H. C. Cornelissen, R. D. Bardgett, M. P. Berg, P. Cipriotti, C. K. Feld, D. Hering, P. Martins da Silva, S. G. Potts, L. Sandin, J. P. Sousa, J. Storkey, D. A. Wardle, and P. A. Harrison. 2010. Towards an assessment of multiple ecosystem processes and services via functional traits. Biodiversity and Conservation 19:2873–2893.

de Bello, F., P. Šmilauer, J. A. F. Diniz-Filho, C. P. Carmona, Z. Lososová, T. Herben, and L. Götzenberger. 2017. Decoupling phylogenetic and functional diversity to reveal hidden signals in community assembly. Methods in Ecology and Evolution 8:1200–1211.

Blankinship, J. C., P. A. Niklaus, and B. A. Hungate. 2011. A meta-analysis of responses of soil biota to global change. Oecologia 165:553–565.

Bobbink, R., K. Hicks, J. Galloway, T. Spranger, R. Alkemade, M. Ashmore, M. Bustamante, S. Cinderby, E. Davidson, F. Dentener, B. Emmett, J. W. Erisman, M. Fenn, F. Gilliam, A. Nordin, L. Pardo, and W. De Vries. 2010. Global assessment of nitrogen deposition effects on terrestrial plant diversity: A synthesis. Ecological Applications 20:30–59.

Byrnes, J. E. K., L. Gamfeldt, F. Isbell, J. S. Lefcheck, J. N. Griffin, A. Hector, B. J. Cardinale, D. U. Hooper, L. E. Dee, and J. Emmett Duffy. 2014. Investigating the relationship between biodiversity and ecosystem multifunctionality: challenges and solutions. Methods in Ecology and Evolution 5:111–124.

Cadotte, M. W. 2017. Functional traits explain ecosystem function through opposing mechanisms. Ecology Letters 20:989–996.

Cadotte, M. W., C. H. Albert, and S. C. Walker. 2013. The ecology of differences: assessing community assembly with trait and evolutionary distances. Ecology Letters 16:1234–1244.

Carmona, C. P., F. de Bello, N. W. H. Mason, and J. Lepš. 2016. Traits without borders: integrating functional diversity across scales. Trends in Ecology and Evolution 31:382–394.

Carmona, C. P., F. de Bello, N. W. H. Mason, and J. Lepš. 2019. Trait probability density (TPD): measuring functional diversity across scales based on TPD with R. Ecology 100:1–8.

Cernansky, R. 2017. The Biodiversity revolution. Nature 546:22–24.

Colin, Y., M. Goberna, M. Verdú, and J. A. Navarro-Cano. 2019. Successional trajectories of soil bacterial communities in mine tailings: The role of plant functional traits. Journal of Environmental Management 241:284–292.

Cornelissen, J. H. C. 1996. An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. The Journal of Ecology 84:573.

Dacal, M., M. Delgado-Baquerizo, J. Barquero, A. A. Berhe, A. Gallardo, F. T. Maestre, and P. García-Palacios. 2022. Temperature increases soil respiration across ecosystem types and soil development, but soil properties determine the magnitude of this effect. Ecosystems 25:184–198.

Delgado-Baquerizo, M., F. T. Maestre, A. Gallardo, M. A. Bowker, M. D. Wallenstein, J. L. Quero, V. Ochoa, B. Gozalo, M. García-Gómez, S. Soliveres, P. García-Palacios, M. Berdugo, E. Valencia, C. Escolar, T. Arredondo, C. Barraza-Zepeda, D. Bran, J. A. Carreira, M. Chaieb, A. A. Conceição, M. Derak, D. J. Eldridge, A. Escudero, C. I. Espinosa, J. Gaitán, M. G. Gatica, S. Gómez-González, E. Guzman, J. R. Gutiérrez, A. Florentino, E. Hepper, R. M. Hernández, E. Huber-Sannwald, M. Jankju, J. Liu, R. L. Mau, M. Miriti, J. Monerris, K. Naseri, Z. Noumi, V. Polo, A. Prina, E. Pucheta, E. Ramírez, D. A. Ramírez-Collantes, R. Romão, M. Tighe, D. Torres, C. Torres-Díaz, E. D. Ungar, J. Val, W. Wamiti, D. Wang, and E. Zaady. 2013. Decoupling of soil nutrient cycles as a function of aridity in global drylands. Nature 502:672–676.

Dias, A. T. C., M. P. Berg, F. de Bello, A. R. Van Oosten, K. Bílá, and M. Moretti. 2013. An experimental framework to identify community functional components driving ecosystem processes and services delivery. Journal of Ecology 101:29–37.

Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, M. Kleyer, C. Wirth, I. Colin Prentice, E. Garnier, G. Bönisch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles, J. Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. Joseph Wright, S. N. Sheremet'ev, H. Jactel, C. Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S. Joswig, A. Günther, V. Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2016. The global spectrum of plant form and function. Nature 529:167–171.

Díaz, S., S. Lavorel, F. de Bello, F. Quétier, K. Grigulis, and T. M. Robson. 2007. Incorporating plant functional diversity effects in ecosystem service assessments. Proceedings of the National Academy of Sciences 104:20684–20689.

Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carré, J. R. G. Marquéz, B.

Gruber, B. Lafourcade, P. J. Leitão, T. Münkemüller, C. Mcclean, P. E. Osborne, B. Reineking, B. Schröder, A. K. Skidmore, D. Zurell, and S. Lautenbach. 2013. Collinearity: a review of methods to deal with it and a simulation study evaluating their performance. Ecography 36:27–46.

Durka, W., and S. G. Michalski. 2012. Daphne: a dated phylogeny of a large European flora for phylogenetically informed ecological analyses. Ecology 93:2297–2297.

Eisenhauer, N., J. Hines, F. Isbell, F. van der Plas, S. E. Hobbie, C. E. Kazanski, A. Lehmann, M. Liu, A. Lochner, M. C. Rillig, A. Vogel, K. Worm, and P. B. Reich. 2018. Plant diversity maintains multiple soil functions in future environments. eLife 7:1–20.

Eivazi, F., and M. A. Tabatabai. 1988. Glucosidases and galactosidases in soils. Soil Biology and Biochemistry 20:601–606.

Eldridge, D. J., M. Delgado-Baquerizo, J. L. Quero, V. Ochoa, B. Gozalo, P. García-Palacios, C. Escolar, M. García-Gómez, A. Prina, M. A. Bowker, D. E. Bran, I. Castro, A. Cea, M. Derak, C. I. Espinosa, A. Florentino, J. J. Gaitán, G. Gatica, S. Gómez-González, W. Ghiloufi, J. R. Gutierrez, E. Gusmán-Montalván, R. M. Hernández, F. M. Hughes, W. Muiño, J. Monerris, A. Ospina, D. A. Ramírez, Y. A. Ribas-Fernández, R. L. Romão, C. Torres-Díaz, T. B. Koen, and F. T. Maestre. 2020. Surface indicators are correlated with soil multifunctionality in global drylands. Journal of Applied Ecology 57:424–435.

Fargione, J. E., and D. Tilman. 2005. Diversity decreases invasion via both sampling and complementarity effects. Ecology Letters 8:604–611.

Finerty, G. E., F. de Bello, K. Bílá, M. P. Berg, A. T. C. Dias, G. B. Pezzatti, and M. Moretti. 2016. Exotic or not, leaf trait dissimilarity modulates the effect of dominant species on mixed litter decomposition. Journal of Ecology 104:1400–1409.

Flynn, D. F. B., N. Mirotchnick, M. Jain, M. I. Palmer, and S. Naeem. 2011. Functional and phylogenetic diversity as predictors of biodiversity-ecosystem-function relationships. Ecology 92:1573–1581.

Galland, T., G. Adeux, H. Dvořáková, A. E-Vojtkó, I. Orbán, M. Lussu, J. Puy, P. Blažek, V. Lanta, J. Lepš, F. Bello, C. Pérez Carmona, E. Valencia, and L. Götzenberger. 2019. Colonization resistance and establishment success along gradients of functional and phylogenetic diversity in experimental plant communities. Journal of Ecology 107:2090–2104.

Garnier, E., J. Cortez, G. Billès, M. Navas, C. Roumet, M. Debussche, G. Laurent, A. Blanchard, D. Aubry, A. Bellmann, C. Neill, and J.-P. Toussaint. 2004. Plant functional markers capture ecosystem properties during secondary succession. Ecology 85:2630–2637.

Goberna, M., J. A. Navarro-Cano, and M. Verdú. 2016. Opposing phylogenetic diversity gradients of plant and soil bacterial communities. Proceedings of the Royal Society B: Biological Sciences 283.

Grace, J. B. 2006. Structural equation modeling and natural systems. Cambridge University Press, Cambridge, UK.

Grace, J. B., and K. A. Bollen. 2005. Interpreting the results from multiple regression and structural equation models. Bulletin of the Ecological Society of America 86:283–295.

Hättenschwiler, S., A. V. Tiunov, and S. Scheu. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 36:191–218.

Haynes, R. J., and R. Naidu. 1998. Influence of lime, fertilizer and manure applications on soil organic matter. Nutrient Cycling in Agroecosystems 51:123–137.

Hector, A., E. Bazeley-White, M. Loreau, S. Otway, and B. Schmid. 2002. Overyielding in grassland communities: Testing the sampling effect hypothesis with replicated biodiversity experiments. Ecology Letters 5:502–511.

Hinsinger, P., C. Plassard, C. Tang, and B. Jaillard. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. Plant and Soil 248:43–59.

Hobbie, S. E. 2015. Plant species effects on nutrient cycling: revisiting litter feedbacks. Trends in Ecology and Evolution 30:357–363.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75:3–35.

Hooper, D. U., and P. M. Vitousek. 1998. Effects of plant composition and diversity on nutrient cycling. Ecological Monographs 68:121–149.

Kandeler, E., and H. Gerber. 1988. Short-Term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils 6:68–72.

Kleyer, M., R. M. Bekker, I. C. Knevel, J. P. Bakker, K. Thompson, M. Sonnenschein, P. Poschlod, J. M. Van Groenendael, L. Klimeš, J. Klimešová, S. Klotz, G. M. Rusch, M. Hermy, D. Adriaens, G. Boedeltje, B. Bossuyt, A. Dannemann, P. Endels, L. Götzenberger, J. G. Hodgson, A. K. Jackel, I. Kühn, D. Kunzmann, W. A. Ozinga, C. Römermann, M. Stadler, J. Schlegelmilch, H. J. Steendam, O. Tackenberg, B. Wilmann, J. H. C. Cornelissen, O. Eriksson, E. Garnier, and B. Peco. 2008. The LEDA Traitbase: A database of life-history traits of the Northwest European flora. Journal of Ecology 96:1266–1274.

Kühn, I., W. Durka, and S. Klotz. 2004. BiolFlor: A new plant-trait database as a tool for plant invasion ecology.

Laliberté, E., and J. M. Tylianakis. 2012. Cascading effects of long-term land-use changes on plant traits and ecosystem functioning. Ecology 93:145–155.

Lamarque, P., S. Lavorel, M. Mouchet, and F. Quetier. 2014. Plant trait-based models identify direct and indirect effects of climate change on bundles of grassland ecosystem services. Proceedings of the National Academy of Sciences 111:13751–13756.

Lavorel, S., and K. Grigulis. 2012. How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. Journal of Ecology 100:128–140.

Lavorel, S., J. Storkey, R. D. Bardgett, F. De Bello, M. P. Berg, X. Le Roux, M. Moretti, C. Mulder, R. J. Pakeman, S. Díaz, and R. Harrington. 2013. A novel framework for linking functional diversity of plants with other trophic levels for the quantification of ecosystem services. Journal of Vegetation Science 24:942–948.

Lefcheck, J. S. 2016. PiecewiseSEM: Piecewise structural equation modelling in R for ecology, evolution, and systematics. Methods in Ecology and Evolution 7:573–579.

Li, W., H. E. Epstein, Z. Wen, J. Zhao, J. Jin, G. Jing, J. Cheng, and G. Du. 2017. Community-weighted mean traits but not functional diversity determine the changes in soil properties during wetland drying on the Tibetan Plateau. Solid Earth 8:137–147.

De Long, J. R., B. G. Jackson, A. Wilkinson, W. J. Pritchard, S. Oakley, K. E. Mason, J. G. Stephan, N. J. Ostle, D. Johnson, E. M. Baggs, and R. D. Bardgett. 2019. Relationships between plant traits, soil properties and carbon fluxes differ between monocultures and mixed communities in temperate grassland. Journal of Ecology 107:1704–1719.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412:72–6.

Maestre, F. T., J. L. Quero, N. J. Gotelli, A. Escudero, V. Ochoa, M. Delgado-Baquerizo, M. García-Gómez, M. A. Bowker, S. Soliveres, C. Escolar, P. García-Palacios, M. Berdugo, E. Valencia, B. Gozalo, A. Gallardo, L. Aguilera, T. Arredondo, J. Blones, B. Boeken, D. Bran, A. A. Conceição, O. Cabrera, M. Chaieb, M. Derak, D. J. Eldridge, C. I. Espinosa, A. Florentino, J. Gaitán, M. Gabriel Gatica, W. Ghiloufi, S. Gómez-González, J. R. Gutiérrez, R. M. Hernández, X. Huang, E. Huber-Sannwald, M. Jankju, M. Miriti, J. Monerris, R. L. Mau, E. Morici, K. Naseri, A. Ospina, V. Polo, A. Prina, E. Pucheta, D. A. Ramírez-Collantes, R. Romão, M. Tighe, C. Torres-Díaz, J. Val, J. P. Veiga, D. Wang, and E. Zaady. 2012. Plant species richness and ecosystem multifunctionality in global drylands. Science 335:214–218.

Mahaut, L., F. Fort, C. Violle, and G. T. Freschet. 2020. Multiple facets of diversity effects on plant productivity: Species richness, functional diversity, species identity and intraspecific competition. Functional Ecology 34:287–298.

Malý, S., J. Královec, and D. Hampel. 2009. Effects of long-term mineral fertilization on microbial biomass, microbial activity, and the presence of r- and K-strategists in soil. Biology and Fertility of Soils 45:753–760.

Mayfield, M. M., and J. M. Levine. 2010. Opposing effects of competitive exclusion on the phylogenetic structure of communities. Ecology Letters 13:1085–1093.

Meyer, S. T., R. Ptacnik, H. Hillebrand, H. Bessler, N. Buchmann, A. Ebeling, N. Eisenhauer, C. Engels, M. Fischer, S. Halle, A. M. Klein, Y. Oelmann, C. Roscher, T. Rottstock, C. Scherber, S. Scheu, B. Schmid, E. D. Schulze, V. M. Temperton, T. Tscharntke, W. Voigt, A. Weigelt, W. Wilcke, and W. W. Weisser. 2018. Biodiversity-multifunctionality relationships depend on identity and number of measured functions. Nature Ecology and

Evolution 2:44–49.

Moreau, D., B. Pivato, D. Bru, H. Busset, F. Deau, C. Faivre, A. Matejicek, F. Strbik, L. Philippot, and C. Mougel. 2015. Plant traits related to nitrogen uptake influence plant-microbe competition. Ecology 96:2300–2310.

Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. Nature 368:734–737.

Navarro-Cano, J. A., M. Goberna, A. Valiente-Banuet, A. Montesinos-Navarro, C. García, and M. Verdú. 2014. Plant phylodiversity enhances soil microbial productivity in facilitation-driven communities. Oecologia 174:909–920.

Navarro-Cano, J. A., B. Horner, M. Goberna, and M. Verdú. 2019. Additive effects of nurse and facilitated plants on ecosystem functions. Journal of Ecology 107:2587–2597.

Navarro-Cano, J. A., M. Verdú, C. García, and M. Goberna. 2015. What nurse shrubs can do for barren soils: rapid productivity shifts associated with a 40 years ontogenetic gradient. Plant and Soil 388:197–209.

Nelson, D. W., and L. E. Sommers. 1996. Total carbon, organic carbon, and organic matter. Page (D. L. Sparks, Ed.) Methods of Soil Analysis, Part 3, Chemical Methods – SSSA Book Series No. 5. Madison, Wisconsin.

Pálková, K., and J. Lepš. 2008. Positive relationship between plant palatability and litter decomposition in meadow plants. Community Ecology 9:17–27.

Pei, G., J. Liu, B. Peng, C. Wang, P. Jiang, and E. Bai. 2020. Nonlinear coupling of carbon and nitrogen release during litter decomposition and its responses to nitrogen addition. Journal of Geophysical Research: Biogeosciences 125:e2019JG005462.

Pérez-Harguindeguy, N., S. Díaz, J. H. C. Cornelissen, F. Vendramini, M. Cabido, and A.

Castellanos. 2000. Chemistry and toughness predict leaf litter decomposition rates over a wide spectrum of functional types and taxa in central Argentina. Plant and Soil 218:21–30.

Pérez-Harguindeguy, N., S. Díaz, S. Lavorel, H. Poorter, P. Jaureguiberry, M. S. Bret-Harte, W. K. Cornwell, J. M. Craine, D. E. Gurvich, C. Urcelay, E. J. Veneklaas, P. B. Reich, L. Poorter, I. J. Wright, P. Ray, L. Enrico, J. G. Pausas, A. C. de Vos, N. Buchmann, G. Funes, F. Quétier, J. G. Hodgson, K. Thompson, H. D. Morgan, H. ter Steege, M. G. A. van der Heijden, L. Sack, B. Blonder, P. Poschlod, M. V. Vaieretti, G. Conti, A. C. Staver, S. Aquino, and J. H. C. Cornelissen. 2013. New Handbook for standardized measurment of plant functional traits worldwide. Australian Journal of Botany 23:167–234.

Pichon, N. A., S. L. Cappelli, S. Soliveres, N. Hölzel, V. H. Klaus, T. Kleinebecker, and E. Allan. 2020. Decomposition disentangled: a test of the multiple mechanisms by which nitrogen enrichment alters litter decomposition. Functional Ecology 34:1485–1496.

van der Plas, F., E. Allan, M. Fischer, F. Alt, H. Arndt, J. Binkenstein, S. Blaser, N. Blüthgen, S. Böhm, N. Hölzel, V. H. Klaus, T. Kleinebecker, K. Morris, Y. Oelmann, D. Prati, S. C. Renner, M. C. Rillig, H. M. Schaefer, M. Schloter, B. Schmitt, I. Schöning, M. Schrumpf, E. F. Solly, E. Sorkau, J. Steckel, I. Steffan-Dewenter, B. Stempfhuber, M. Tschapka, C. N. Weiner, W. W. Weisser, M. Werner, C. Westphal, W. Wilcke, and P. Manning. 2019. Towards the development of general rules describing landscape heterogeneity–multifunctionality relationships. Journal of Applied Ecology 56:168–179.

R Development Core Team. 2018. R: A language and environment for statistical computing. Page (A. R Foundation for Statistical Computing, Vienna, Ed.). R Foundation for Statistical Computing, Vienna, Austria.

Rao, C. R. 1982. Diversity and dissimilarity coefficients: A unified approach. Theoretical

Population Biology 21:24–43.

Roscher, C., J. Schumacher, J. Baade, W. Wilcke, G. Gleixner, W. W. Weisser, B. Schmid, and E.-D. Schulze. 2004. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic and Applied Ecology 5:107–121.

Sardans, J., J. Peñuelas, and M. Estiarte. 2008. Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland. Applied Soil Ecology 39:223–235.

Sardans, J., A. Rivas-Ubach, and J. Peñuelas. 2012. The C:N:P stoichiometry of organisms and ecosystems in a changing world: A review and perspectives. Perspectives in Plant Ecology, Evolution and Systematics 14:33–47.

Scherer-Lorenzen, M., C. Palmborg, A. Prinz, and E. D. Schulze. 2003. The role of plant diversity and composition for nitrate leaching in grasslands. Ecology 84:1539–1552.

Shipley, B. 2009. Confirmatory path analysis in a generalized multilevel context. Ecology 90:363–368.

Shipley, B. 2013. The AIC model selection method applied to path analytic models compared using a d-separation test. Ecology 94:560–564.

Sinsabaugh, R. L., C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. Crenshaw, A. R. Contosta, D. Cusack, S. Frey, M. E. Gallo, T. B. Gartner, S. E. Hobbie, K. Holland, B. L. Keeler, J. S. Powers, M. Stursova, C. Takacs-Vesbach, M. P. Waldrop, M. D. Wallenstein, D. R. Zak, and L. H. Zeglin. 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11:1252–1264.

Tabatabai, M. A., and J. M. Bremner. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biology and Biochemistry 1:301–307.

Tilman, D., and J. a. Downing. 1994. Biodiversity and stability in grasslands. Nature 367:363–365.

Valencia, E., F. de Bello, T. Galland, L. Götzenberger, J. Lepš, J. Durán, and C. P. Carmona. 2022. Effects of functional and phylogenetic diversity on the temporal dynamics of soil N availability. Plant and Soil 472:629-640.

Valencia, E., T. Galland, C. P. Carmona, M. Goberna, L. Götzenberger, J. Lepš, M. Verdú, P. Macek, and F. de Bello. 2022. Data from "The functional structure of plant communities drives soil functioning via changes in soil abiotic properties". figshare. Dataset. https://doi.org/10.6084/m9.figshare.16536357.v1

Valencia, E., N. Gross, J. L. Quero, C. P. Carmona, V. Ochoa, B. Gozalo, M. Delgado-Baquerizo, K. Dumack, K. Hamonts, B. K. Singh, M. Bonkowski, and F. T. Maestre. 2018. Cascading effects from plants to soil microorganisms explain how plant species richness and simulated climate change affect soil multifunctionality. Global Change Biology 24:5642–5654.

Venables, W., and B. Ripley. 2002. Modern applied statistics with S. Fourth. Springer, New York, NY, New York.

de Vries, F. T., P. Manning, J. R. B. Tallowin, S. R. Mortimer, E. S. Pilgrim, K. A. Harrison, P. J. Hobbs, H. Quirk, B. Shipley, J. H. C. Cornelissen, J. Kattge, and R. D. Bardgett. 2012. Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities. Ecology Letters 15:1230–1239.

Wardle, D. A., R. D. Bardgett, J. N. Klironomos, H. Setälä, W. H. Van Der Putten, and D. H. Wall. 2004. Ecological linkages between aboveground and belowground biota. Science

304:1629–1633.

Webb, C. O., D. D. Ackerly, M. A. Mcpeek, and M. J. Donoghue. 2002. Phylogenetics and community ecology. Annual review of ecology and systematics 33:475–505.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M.-L. Navas, Ü. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428:821–827.

Zavaleta, E. S., J. R. Pasari, K. B. Hulvey, and G. D. Tilman. 2010. Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. Proceedings of the National Academy of Sciences of the United States of America 107:1443–6.

## Figure captions

**Figure 1**: Hypothetical relationships between fertilization, plant community functional and phylogenetic structure (FD = functional diversity, PD = phylogenetic diversity, CWMs = community weighted means), plant-related ecosystem functions (EFs), soil abiotic conditions, soil fertility and soil microbial activity. Fertilization treatment and plant structure (FD, PD and CWMs) could affect directly microbial activity (Malý et al. 2009, Valencia et al. 2018, Le Bagousse-Pinguet et al. 2019). However, they could also affect indirectly (i.e. cascading effect) soil microbial activity via changes in plant-related EFs, soil abiotic conditions and soil fertility. Both fertilization and plant structure may directly alter plant-related EFs, soil abiotic properties and soil fertility (arrows "c" to "h"; Bobbink et al. 2010, Laliberté and Tylianakis 2012, Sardans et al. 2012, Navarro-Cano et al. 2014, 2019, Goberna et al. 2016, Li et al. 2017, Eldridge et al. 2020, Pichon et al. 2020, Pei et al. 2020, Valencia et al. 2022). For instance, root exudates, productivity, litter decomposition, and soil humidity are variables which may depend on the functional traits of plants inhabiting a given community (de Bello et al. 2010). However, fertilization and plant structure may also affect soil abiotic properties via changes in plant aboveground biomass, litter decomposability and / or soil fertility (arrows "i" to "k"; Hättenschwiler et al. 2005, Sinsabaugh et al. 2008, Blankinship et al. 2011, Eldridge et al. 2020). Typically, increases in organic matter (through root exudates or litter deposition arrows "h" and "i", respectively) lead to concurrent increases in acidity and electrical conductivity (more organic acids are released into the soil solution) and soil moisture (Hinsinger et al. 2003). Finally, the right part of the figure shows how the plant-related EFs, soil abiotic conditions and soil fertility affect soil microbial activity (arrows "h", to "j"; Sinsabaugh et al. 2008, Delgado-Baquerizo et al. 2013, Dacal et al. 2022).

**Figure 2: A)** PCA on 19 species pool populations in trait space. Each color represents one species. Red arrows represent traits: H = plant height, LDMC = leaf dry matter content, SLA = specific leaf area, P = leaf phosphorus content, CN = carbon to nitrogen ratio in leaves. **B)** PCA on ecosystem property variables. The colors represent groups of ecosystem property variables: navy blue for plant biomass (Biomass sown and Biomass weed for sown or invasive species respectively), turquoise for litter decomposability, red for soil abiotic properties (pH, EC = electrical conductivity, GH = gravimetric humidity), green for soil fertility (TOC = total organic carbon, TN = total nitrogen, P = phosphorus and K = potassium) and gold for soil microbial activity (rslope = slope of the soil $CO_2$-C accumulation curve, GA = β-glucosidase activity, PA = alkaline phosphatase activity, UA = urease activity).

**Figure 3:** Effect of fertilization and plant community functional and phylogenetic structure (CWMs, FD, PD) on individual ecosystem functions/properties and averaged multifunctionality indices. Each line represents the final linear model for a given response variable after forward selection. The adjusted coefficient of determination of the models are given in parenthesis next to each response variable. We show the averaged parameter estimates (standardized regression coefficients) of model predictors and the associated 95% confidence intervals. The colors represent groups of ecosystem function variables: navy blue for plant biomass productivity and its diversity effect decomposition (biom. sown and biom. weed for biomass of sown and invasive species respectively, NetEffect = net biodiversity effect, Sel = selection effect, Comp = complementarity effect), turquoise for litter decomposability (Decomp), red for soil abiotic properties (pH, EC = electro conductivity, GH = gravimetric humidity), green for soil nutrient pool (TOC = total organic carbon, TN = total nitrogen, P = phosphorus and K = potassium), gold for soil microbial activity (rslope = slope of the soil $CO_2$-C accumulation curve, GA = β-

glucosidase activity, PA = alkaline phosphatase activity, UA = urease activity) and black for averaged multifunctionality indices (MultF. all and MultF. soil). In addition, light grey colors are coefficients selected in the final model, but not significant. Fert = fertilization treatment, FD = functional diversity, PD = phylogenetic diversity, CWM-PCA1 = CWM obtained with the PCA of trait values (axis 1) and the observed biomass; high CWM-PCA1 is related to taller species and higher LDMC, CWM-PCA2 = CWM obtained with the PCA of trait values (axis 2) and the observed biomass, high CWM-PCA2 is related to higher leaf C:N ratio, lower SLA and fewer legume species.

**Figure 4:** Structural equation models showing the effects of fertilization and plant community functional and phylogenetic structure on soil microbial activity, mediated by decomposability and soil abiotic properties (there are four piecewise SEMs superposed, one per soil microbial activity variable. See Appendix S1: Figure S10 to visualize each of the piecewise SEMs separately). The variances explained for each individual model are given in the response variable boxes. Blue and red arrows describe positive and negative effects respectively, solid lines are significant paths ($p$-value $< 0.05$) and dashed lines are marginally significant paths ($p$-value $< 0.1$). Non-significant paths are not represented for clarity. The width of the arrows is proportional to the strength of the relationship. See Appendix S1: Table S3 for all coefficients and global goodness-of-fit measures for individual models. FD = functional diversity, PD = phylogenetic diversity, CWM-PCA1 = CWM obtained with the PCA of trait values (axis 1) and the observed biomass; high CWM-PCA1 is related to taller species and higher LDMC, CWM-PCA2 = CWM obtained with the PCA of trait values (axis 2) and the observed biomass, high CWM-PCA2 is related to higher leaf C:N ratio, lower SLA and fewer legume species, Litter decomp. = litter decomposability, MultF-NutPool =

aggregated soil nutrient pool (Mean of the four Z-transformed nutrient pool variables: total organic carbon, total nitrogen, phosphorus and potassium), EC = electro conductivity, GH = gravimetric humidity, GA = β-glucosidase activity, UA = urease activity, PA = alkaline phosphatase activity, rslope = slope of the soil $CO_2$-C accumulation curve.

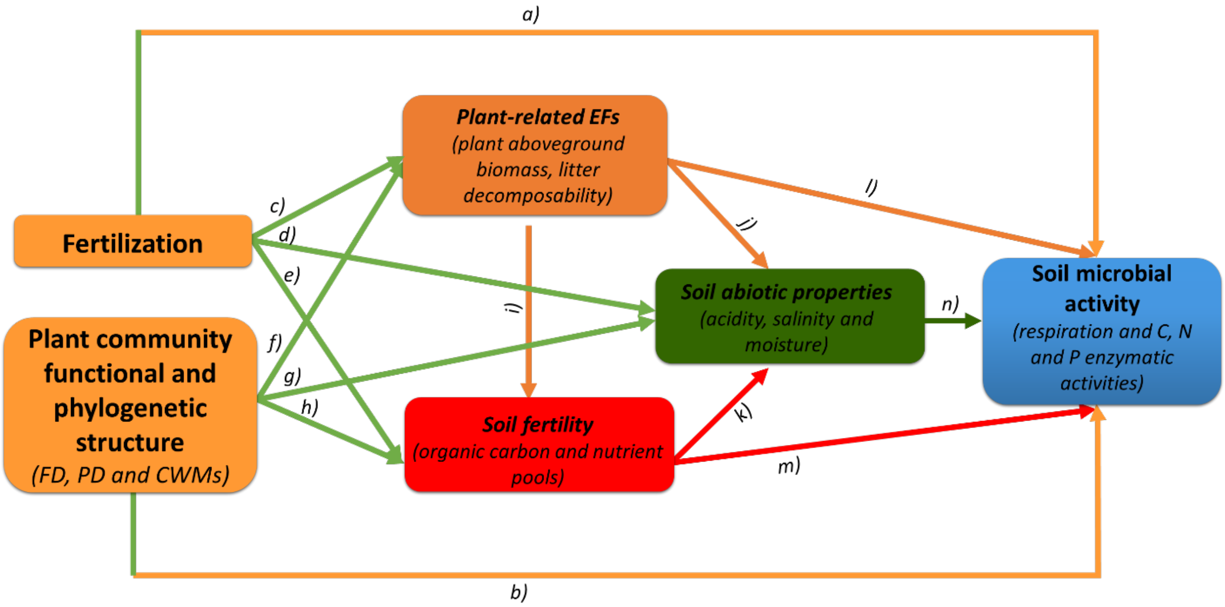
a)
Plant-related EFs
(plant aboveground biomass, litter decomposability)
Fertilization
c)
d)
e)
l)
j)
i)
Soil abiotic properties
(acidity, salinity and moisture)
n)
Soil microbial activity
(respiration and C, N and P enzymatic activities)
Plant community functional and phylogenetic structure
(FD, PD and CWMs)
f)
g)
h)
Soil fertility
(organic carbon and nutrient pools)
k)
m)
b)

**Figure 1.**

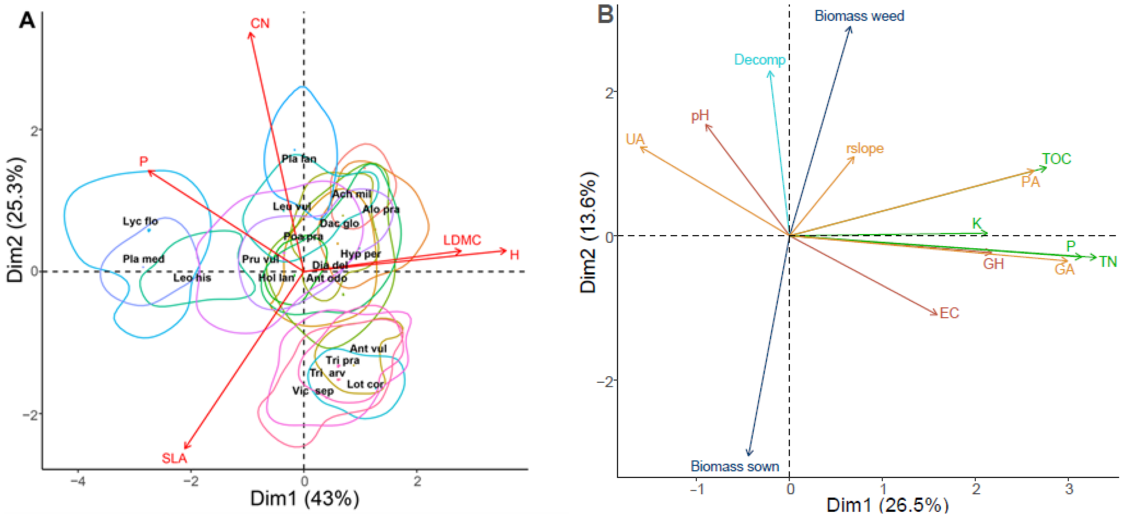
A
CN
P
LDMC
H
SLA
Pla fan
Ach mil
Leu vul
Alo pra
Lyc flo
Dac glo
Poa pra
Pla med
Pru vul
Hyp per
Dia del
Leo his
Hol lan
Ant odo
Ant vul
Tri pra
Tri arv
Lot cor
Vic sep
Dim2 (25.3%)
Dim1 (43%)
2
0
−2
−4
−2
0
2
B
Biomass weed
Decomp
pH
UA
rslope
TOC
PA
K
P
TN
GH
GA
EC
Biomass sown
Dim2 (13.6%)
Dim1 (26.5%)
2
0
−2
−1
0
1
2
3

**Figure 2.**

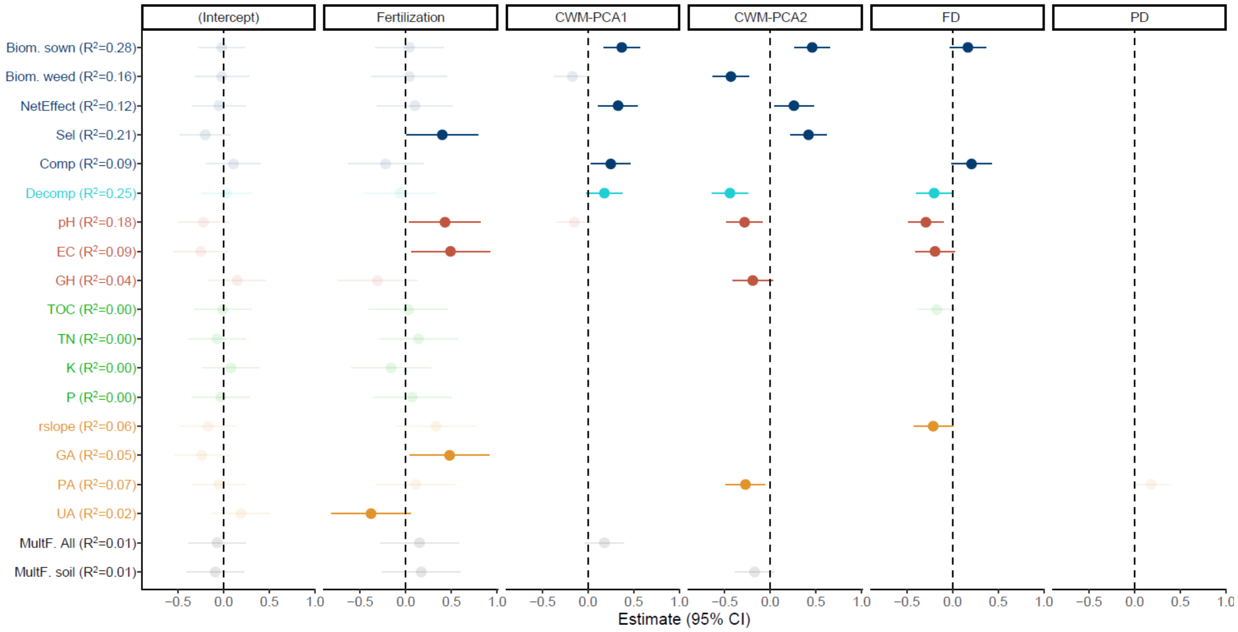
(Intercept)
Fertilization
CWM-PCA1
CWM-PCA2
FD
PD
Biom. sown (R²=0.28)
Biom. weed (R²=0.16)
NetEffect (R²=0.12)
Sel (R²=0.21)
Comp (R²=0.09)
Decomp (R²=0.25)
pH (R²=0.18)
EC (R²=0.09)
GH (R²=0.04)
TOC (R²=0.00)
TN (R²=0.00)
K (R²=0.00)
P (R²=0.00)
rslope (R²=0.06)
GA (R²=0.05)
PA (R²=0.07)
UA (R²=0.02)
MultF. All (R²=0.01)
MultF. soil (R²=0.01)
−0.5 0.0 0.5 1.0
Estimate (95% CI)

**Figure 3.**

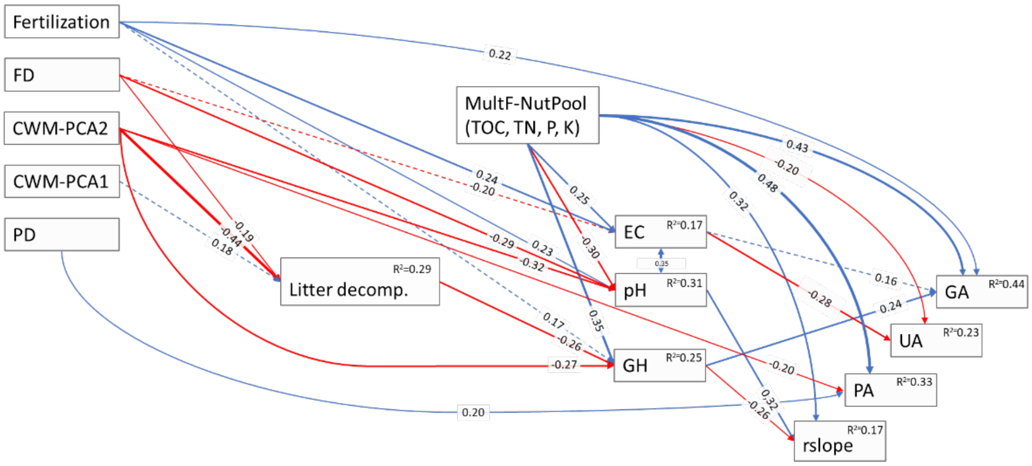
Fertilization
FD
CWM-PCA2
CWM-PCA1
PD
MultF-NutPool (TOC, TN, P, K)
0.22
0.43
-0.20
0.48
0.32
0.24
-0.20
0.25
-0.19
-0.44
0.18
-0.29
0.23
-0.32
-0.30
EC R²=0.17
0.35
pH R²=0.31
0.16
GA R²=0.44
-0.28
0.24
UA R²=0.23
Litter decomp. R²=0.29
0.17
-0.26
0.35
-0.27
GH R²=0.25
-0.20
PA R²=0.33
0.32
-0.26
rslope R²=0.17
0.20

**Figure 4.**