Soil Biology and Biochemistry

Litter diversity accelerates labile carbon but slows recalcitrant carbon decomposition

--Manuscript Draft--

Manuscript Number:	SBB19175R1
Article Type:	Research Paper
Keywords:	Biodiversity-Ecosystem functioning; Carbon fractions; functional diversity; Litter diversity; Litter quality; Litter mixture
Corresponding Author:	François-Xavier Joly University of Vienna: Universitat Wien Vienna, AUSTRIA
First Author:	Lifeng Wang
Order of Authors:	Lifeng Wang
	Yu Zhou
	Yamei Chen
	Zhenfeng Xu
	Jian Zhang
	Yang Liu
	François-Xavier Joly
Manuscript Region of Origin:	CHINA
Suggested Reviewers:	Jake Grossman Assistant Professor, Swarthmore College jgrossm1@swarthmore.edu Expert on litter diversity effect on contrasted litter fractions; He published the only other similar study to date, in Ecological Monographs.
	Samantha K Chapman Villanova University samantha.chapman@villanova.edu Prof. Chapman is an expert on litter diversity effect on decomposition
	Mathieu Santonja Aix-Marseille Université: Aix-Marseille Universite mathieu.santonja@imbe.fr Dr. Santonja is an expert on litter diversity on decomposition

Accepted refereed manuscript of: Wang L, Zhou Y, Chen Y, Xu Z, Zhang J, Liu Y & Joly F (2022) Litter diversity accelerates labile carbon but slows recalcitrant carbon decomposition. *Soil Biology and Biochemistry*, 168, p. 108632.
https://doi.org/10.1016/j.soilbio.2022.108632

Litter diversity accelerates labile carbon but slows recalcitrant carbon decomposition

Lifeng Wang[a], Yu Zhou[a], Yamei Chen[b], Zhenfeng Xu[a], Jian Zhang[a*], Yang Liu[a*], François-Xavier Joly[c]

[a] Long-term Research Station of Alpine Ecosystems, Key Laboratory of Ecological Forestry Engineering of Sichuan Province, Institute of Ecology & Forests, Sichuan Agricultural University, Chengdu 611130, China

[b] Key Laboratory of Southwest China Wildlife Resources Conservation, China West Normal University, Ministry of Education, Nanchong, Sichuan 637009, China

[c] Division of Terrestrial Ecosystem Research, Center for Microbiology and Environmental Systems Science, University of Vienna, Djerassiplatz 1, 1030, Vienna, Austria

[*] Corresponding author: Jian Zhang, sicauzhangjian@163.com, Yang Liu, sicauliuyang@163.com;

Abstract

In biodiverse ecosystems, leaf litter of different plant species decomposes in mixtures, for which decomposition rates notoriously deviate from that expected from monospecific treatments. Despite important research efforts in past decades, these litter diversity effects remain difficult to predict. We hypothesized that this is due to a focus on bulk litter decomposition, while different carbon fractions constituting the litter may respond differently to litter diversity, thereby blurring the overall response. To test this hypothesis, we determined how the decomposition of (i) soluble compounds, (ii) cellulose, and (iii) lignin responded to litter mixing in a 3.5-year field experiment in an alpine forest. We found that the decomposition of soluble compounds and cellulose in mixtures was faster than expected from monospecific treatments, while that of lignin was slower. These deviations from expected decomposition rates of each litter carbon fraction were driven by different aspects of the litter functional diversity. This suggests that different mechanisms operating on distinct litter fractions lead to synergistic and antagonistic interactions that simultaneously affect bulk litter decomposition. Furthermore, the magnitude of these fraction-specific deviations from expected decomposition rates consistently decreased throughout decomposition. Considering the response of litter fractions and their temporality, rather than focusing on bulk litter thus seems critical to evaluate the response of decomposition to plant diversity and identify underlying mechanisms.

Keywords

Biodiversity–Ecosystem functioning; Carbon fractions; Functional diversity; Litter diversity; Litter quality; Litter mixture;

1. Introduction

Plant litter decomposition is a fundamental ecosystem process, determining carbon (C) cycling and soil fertility. It is predominantly driven by climatic conditions, litter physicochemical characteristics, and the community of decomposer organisms (Berg and McClaugherty, 2020). In biodiverse ecosystems, it has also been repeatedly observed that leaf litter decomposes differently as a group of different plant species than when litter of component species decomposes separately (Gartner and Cardon, 2004). This indicates that important interactions between co-decomposing litter can accelerate (i.e. synergistic effect) or retard (i.e. antagonistic effect) the decomposition of the entire litter mixture (Hättenschwiler et al., 2005; Chapman and

Koch, 2007; Gessner et al., 2010). These substantial litter diversity effects on decomposition may importantly affect biogeochemical cycles, but their magnitude and direction are difficult to predict (Kou et al., 2020), limiting the inclusion of litter diversity in decomposition models.

Several mechanisms have been reported as drivers of these litter diversity effects. These include (i) transfers of nutrients from nutrient-rich litter to nutrient-poor litter that facilitates the decomposition of the latter and that of the overall mixtures (Schimel and Hättenschwiler, 2007; Handa et al., 2014), (ii) improved microenvironmental conditions from one litter with high water-holding capacity that benefit the decomposition of the whole mixture (Makkonen et al., 2013), (iii) complementary resource use by decomposer organisms (Vos et al., 2013), or (iv) presence of specific compounds such as secondary metabolites in one litter that can favor or limit decomposer activity and alter the decomposition of the whole mixture (Schimel et al., 1998). Since these mechanisms rely on differences in litter characteristics amongst co-decomposing litter (e.g. nutrient concentrations, water-holding capacity, secondary metabolite concentrations), most studies to date trying to predict litter diversity effects (expressed as ‘relative mixing effect’, i.e. the relative difference in decomposition between that observed in the mixture and that predicted based on component litter species decomposing separately) used indices of litter characteristic dissimilarity (Barantal et al., 2014; Tardif and Shipley, 2015; Kuebbing and Bradford, 2019). Yet, no dominant characteristic dissimilarity has emerged as a driver of litter diversity effects. This may be due to the fact that these studies focused on bulk litter mass loss as a proxy of decomposition, while the different C fractions constituting the litter may respond differently to these interactions, blurring the overall response.

Leaf litter is predominantly made of lignin, cellulose, hemicellulose and water-soluble compounds, which have contrasting chemistries and thus decompose differently. Soluble compounds, as the most labile compounds, are primarily lost by water passage through the litter during the early stages of decomposition. In turn, cellulose and hemicellulose are degraded by microorganisms during early and mid-decomposition stages, while lignin is decomposed by specialist microorganisms at later decomposition stages (Shipley and Tardif, 2021; Berg and McClaugherty, 2020). Owing to these chemical differences, these litter C fractions may respond differently to changes in litter functional diversity. One hint in this direction derives from an important discovery on litter diversity effects (Handa et al., 2014), which reported that positive litter diversity effects occurred when nitrogen (N) was transferred from litter of N-fixing plants to readily decomposable litter, but not to slowly decomposing litter. A potential underlying mechanism is that N transfer accelerates the decomposition of the more labile litter C fractions by lifting N limitation, while it does not affect the decomposition of the more slowly decomposing fractions. The responses of these different litter C fractions to litter mixing may differ importantly, but they have rarely been evaluated. We know of only one study that examined the responses of these different litter C fractions to litter functional diversity, which found in a two-year experiment, that litter mixing slowed the mass loss of the most labile litter C fraction but did not affect the decomposition of more recalcitrant fractions (Grossman et al., 2020). This contrasts with the expectation that readily decomposable litter C fractions benefit more from litter-mixing compared to more recalcitrant fractions, and the mechanisms leading to this slower decomposition of the labile fraction are unknown. Yet, with only one study available to date, the effect of litter diversity on the decomposition of different litter C fractions remain unpredictable. Additionally, owing to the distinct temporal dynamics of these litter C fractions, the response of their decomposition to litter-mixing may vary throughout decomposition, but this temporal variation remains unexplored.

Here, we contributed to filling this knowledge gap by evaluating how litter diversity control the decomposition of bulk litter and of the litter fractions (soluble compounds, cellulose, and lignin) in an alpine forest, in a 42-month (3.5-year) decomposition experiment. To do so, we followed the decomposition of bulk litter and litter fraction in mixtures of litter from multiple plant functional types and compared it to that expected from single plant functional type treatments. We hypothesize (i) that litter mixtures decomposed faster than component litter decomposing separately, (ii) that this was driven by a faster decomposition of the labile litter C fraction rather than by a change in recalcitrant litter C fraction decomposition, (iii) that litter-mixing effects on different litter C fractions are related to different aspects of litter characteristic dissimilarity, and (iv) that litter-mixing effects on different litter C fractions peak at different decomposition stages.

2. Methods

The experiment was conducted in an alpine forest at the Long-Term Research Station of Alpine Forest Ecosystems at Zhegu Mountain, China (31°51′N, 102°41′E; 3900 ~ 4000m a.s.l.). The climate is characterized with a mean annual precipitation and temperature of 802 mm and 2.9 °C (2013~2019) respectively. The soil is a Histosol (pH = 5; C/N = 21; Wang et al., 2021a). Six plant functional types dominate the vegetation, including evergreen conifers (*Abies faxoniana* Rehd.), evergreen shrubs (*Rhododendron lapponicum* (L.) Wahl), deciduous shrubs (*Sorbus rufopilosa* Schneid. and *Salix paraplesia* Schneid.), ferns (*Cystopteris moupinensis* Franch.), graminoids (*Deyeuxia scabrescens* Griseb. and *Poa crymophila* Keng.) and forbs (*Epilobium angustifolium* (L.) Scop and *Ligularia sagittal* Maxim.).

To evaluate the effect of litter diversity on the long-term decomposition of distinct litter C fractions, we followed the decomposition of litter from six plant functional types in litterbags, separately and in combination, during a 3.5 year field incubation. Monocultures consisted of litter from the six plant functional types separately. Mixtures consisted of all possible plant functional type combinations with six and five plant functional types, including one mixture with all six plant functional types, and six mixtures with five plant functional types. This lead to a total of 13 litter treatments. Each litter treatment was placed to decompose in 10 plots of 5 × 5 m at the study site, organized in two parallel transects each containing five replicate plots. This design aimed to capture the microenvironmental variability of this ecosystem prone to high heterogeneity. In each plot, five replicates of each litter treatment were incubated and harvested after 146, 330, 513, 669, and 1279 days. This led to 13 litter treatments × 2 transects × 5 plots × 5 harvests = 650 litterbags.

We collected freshly-senesced leaf litter of each plant functional type (evergreen conifers, evergreen shrubs, deciduous shrubs, ferns, graminoids and forbs) in October 2015 from the forest floor and dried it at room temperature. Litterbags (20 × 25 cm) were constructed from polyethylene fabrics of 1 × 1 mm mesh sizes allowing access to microorganisms, microfauna, and some mesofauna. Litterbags were filled with 12 g of air-dried litter, with equal proportions of each plant functional type in the respective litter mixtures. When plant functional types consisted of two species (deciduous shrubs, forbs, and graminoids), litter of each species was placed in equal proportions. We applied an air-dry/oven-dry conversion factor, determined from subsamples of each litter dried at 65 °C for 48 h, to initial litter masses. We corrected initial litter masses for litter losses during setup using transport litterbags (Wang et al., 2021b).

Upon harvest, decomposed litter from litterbags was manually cleaned of adhering soil particles and foreign plant material, dried at 65 °C, weighed, and ground using a fine powder with a ball mill. The relative abundance of water-soluble compounds and non-polar extractables (simple sugars, water-soluble phenolics and fats, waxes, and oils; referred to as "Solubles" hereafter), acid-hydrolyzable compounds (cellulose and hemicellulose; referred to as "Cellulose" hereafter) and acid-unhydrolyzable compounds (lignin, less-soluble condensed tannins, cutins, suberin, and surface waxes, referred to as "Lignin" thereafter) in the initial and decomposed litter samples were determined as Wang et al. (2021b). For each sample, mass losses (Table S1 for monocultures; Table S2 for mixtures) were computed for bulk litter and for each litter C fraction (solubles, cellulose, and lignin) as follows:

$$[(M_i \times C_i) - (M_t \times C_t)] / (M_i \times C_i) \times 100\%, \text{ (Equation 1)}$$

where M_i and M_t represent the oven-dried litter mass initially and at time t, respectively. For each litter C fraction mass loss calculations, C_i and C_t represent the relative contribution of the corresponding litter C fraction in the total litter mass, initially and at time t, respectively. Decomposition rates (k constants) were then computed for bulk litter mass loss and litter fraction mass losses, by fitting three alternative models, including single-exponential, double-exponential and asymptotic decomposition models (Grossman et al., 2020; Wang et al., 2021b). Based on the corrected Akaike Information Criterion, single-exponential decay models fit best to describe bulk litter, solubles, cellulose, and lignin decomposition. Hereafter, we report k constants from single exponential models (Table S1 for monocultures; Table S2 for mixtures). To determine the effect of litter mixing on the decomposition of bulk litter and litter C fractions, we computed, for each litter mixture, the relative mixing effects (RMEs) on bulk litter/litter C fraction mass losses at each harvest, and on bulk litter/litter C fraction decomposition rate (*k* constants) as follows (Wardle et al., 1997):

$$[(\text{observed value} - \text{expected value}) / \text{expected value}] \times 100\%. \text{ (Equation 2)}$$

where the observed value is the observed mass loss or decomposition rate (of the bulk litter/litter C fractions) in a given litter mixture, and where the expected value is the average mass loss or decomposition rate (of the bulk litter/litter C fractions) of the bulk litter/litter C fractions of the component plant functional types decomposing singly (Table S2).

To determine the control of litter trait diversity on the RMEs on bulk litter/litter C fraction decomposition rates, we analyzed the physicochemical characteristics of all plant functional litter type. We measured twelve chemical traits, including total C, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn), and zinc (Zn), leachate C, leachate N, leachate P, and two physical traits including the specific leaf area (SLA) and water holding capacity (WHC) (Wang et al., 2021a, b). We also computed the C/N, C/P, N/P, Lignin/N, and Lignin/P ratios as litter traits. Using functional type-specific litter traits (Table S3) and the relative abundance of litter from each functional type in litter mixtures, we measured litter functional dissimilarity using Rao's quadratic entropy as follows:

$$\text{Litter FD}_{ik} = \sum_{i=1}^{N} \sum_{k=1}^{N} p_i \times p_k \times \text{distance}_{ik} \text{ , (Equation 3)}$$

where p_i and p_k is the relative abundance (masses) of functional type *i* and *k*, respectively, and distance_{ik} the trait dissimilarity based on pair-wise Euclidean distance between species *i* and *k* in the functional trait space.

To identify changes in RME throughout decomposition, we evaluated the relationship between RMEs on bulk litter/litter fractions mass loss after 146, 330, 513, 669, and 1279 days of exposure in the field, and bulk litter mass loss using simple linear regressions across all mixtures. To synthesize the multiple indices of litter functional diversity (Raos), we used principal component analyses (PCA) including all Raos, separately. Then, we used Pearson correlations to visualize relations between litter functional diversity parameters (PC1, PC2, PC3, and PC4 scores) and RME on bulk litter/litter C fraction decomposition rates (*k* constant). We also used multiple mixed-effects linear regressions to explore relationship between RME on bulk litter/litter fraction decomposition rates and species coordinates on the first four axes of each PCA (the fixed predictor), with plots nested within transects included as random variables. We also fitted simple mixed-effects linear regressions for RMEs on bulk litter/litter C fraction decomposition rates (*k* constants) as a function of PCA axes with the maximal *t* value in multiple mixed linear regressions. All data were checked for normal distribution and homoscedasticity of residuals, and all analyses were performed using R version 4.0.2.

3. Results

Across litter mixtures, bulk litter mass losses were lower than expected based on single plant functional type treatments, with an average RME of -5.62% across harvests. The magnitude of RMEs on litter mass losses did not significantly change with time ($P = 0.87$; Fig. 1a), resulting in an overall antagonistic effect on *k* constants of -14.88%, ranging from -20.86% for the mixture with all functional types, to -6.43% for the mixture without forbs (Fig. 1a). Soluble losses were higher than expected based on single plant functional type treatments, with an average RME of 13.55% across harvests. The magnitude of RMEs on soluble losses decreased with time ($P < 0.05$; Fig. 1b), switching from positive to null, with an overall synergistic effect on *k* constants of 10.32%, ranging from 3.94% for the mixture with evergreen conifers,-EC to 17.29% for the mixture without deciduous shrubs (Fig. 1b). Cellulose losses were higher than expected based on single plant functional type treatments, with an average RME of 28.24% across harvests. The magnitude of RMEs on cellulose losses decreased with time ($P < 0.05$; Fig. 1c), switching from positive to null, with an overall synergistic effect on *k* constants of 9.64%, ranging from -11.05%

for the mixture without deciduous shrubs, to 32.6% for the mixture without evergreen shrubs (Fig. 1c). Lignin losses were lower than expected based on single plant functional type treatments, with an average RME of -67.35% across harvests. The magnitude of RMEs on lignin losses decreased with time ($P < 0.05$; Fig. 1d), switching from negative to null, with an overall antagonistic effect on *k* constants of -47.32%, ranging from -11.05% for the mixture without deciduous shrubs, to 32.6% for the mixture without evergreen shrubs (Fig. 1d).

The RMEs on *k* constants of bulk litter increased with the second litter functional diversity PC axis (PC2, $P < 0.01$; Fig. 2a, 3a), which is positively related to dissimilarity in litter lignin, Zn contents and N/P, and negatively related to dissimilarity in litter C, leachate C, leachate P, soluble C, K, Na, Ca, Mg, Mn, C/N, C/P, and lignin/P (Fig. 2a), and all first four litter functional diversity PC axes accounted for 10.94% of overall variance (Table S4). The RMEs on *k* constants of solubles increased with the third litter functional diversity PC axis (PC3; $P < 0.05$; Fig. 2b, 3b), which is positively related to dissimilarity in litter WHC, SLA, leachate C, solubles, cellulose, Mg, and N/P, and negatively related to dissimilarity in litter P, leachate N, leachate P, Na, Ca, and Zn (Fig. 2b), and all first four litter functional diversity PC axes accounted for 6.64% of overall variance (Table S4). The RMEs on *k* constant of cellulose particularly increased with the second litter functional diversity PC axes (PC2; $P < 0.001$; Fig. 2a, 3c), and all first four litter functional diversity PC axes accounted for 32.94% of overall variance (Table S4). The RMEs on *k* constant of lignin particularly increased with the first litter functional diversity axes (PC1; $P < 0.001$; Fig. 2a, 3d), which is positively related to dissimilarity in litter WHC, N, P, leachate P, lignin, Mn, C/N, C/P, N/P, lignin/N, and lignin/P, and negatively related to dissimilarity in litter C, Na, Ca, and Mg (Fig. 2a), and all first four litter functional diversity PC axes accounted for 26.26% of overall variance (Table S4).

4. Discussion

With synergistic effects of litter mixing on the decomposition of soluble and cellulose fractions and antagonistic effects for the lignin fraction, our results show clear contrasted responses of different fractions to litter diversity depending on their lability. This finding indicates that important litter diversity effects occurring on different litter fractions can be overlooked in classical litter diversity studies that focus on bulk litter decomposition. The synergistic effects of litter mixing on the soluble and cellulose fractions support our second hypothesis that more labile fractions are positively affected by litter diversity. Indeed, this is in line for instance with the observation from a multi-biome experiment that litter diversity effects are driven by a higher decomposition of readily degradable litter in litter mixtures (Handa et al., 2014). However, our results contrast with those from the only other study to our knowledge that examined the response of litter C fraction decomposition to litter mixing (Grossman et al., 2020), who reported antagonistic effects for labile litter (soluble compounds and hemicellulose), and no effect on the recalcitrant litter. Our study and that of Grossman et al. (2020) differed in several factors, such as study ecosystem, plant functional types and mixtures thereof, and duration of incubation, all of which can affect the direction and magnitude of litter diversity effects (Jonsson and Wardle, 2008; Chen et al., 2021), and may thus have led to the contrasting results. However, given the paucity of data available at this stage, it is difficult to put forward specific reasons and associated mechanisms for these contrasting results, and further studies will be needed to identify general patterns of specific litter carbon fraction response to litter-mixing. Interestingly, in contrast to our first hypothesis, we did not report synergistic on bulk litter decomposition, despite observed synergistic effects on labile fraction in line with our second hypothesis. This indicates that the antagonistic effects on the recalcitrant fraction were more important, leading to overall antagonistic effects on bulk litter decomposition.

Our analysis of the correlations between litter diversity effects (RMEs) and litter functional dissimilarity also suggests that litter diversity effects on the decomposition of different litter C fractions are driven by contrasted aspects of litter traits dissimilarity, in line with our third hypothesis. Interestingly, RMEs on the soluble fraction were positively related to dissimilarity in litter water-holding capacity among co-decomposing litter types, which could increase the litter moisture for the entire litter mixture and favor leaching of water-soluble compounds. A link between dissimilar in water-holding capacity and bulk litter decomposition was previously reported (Makkonen et al., 2013), and attributed to the mechanism of ‘improved microenvironmental conditions’ which is assumed to favor decomposer activity (Hättenschwiler et al., 2005). Our separation of bulk litter into litter C fractions suggests that this mechanism of ‘improved microenvironmental conditions’ may actually affect decomposition by favoring the leaching of water-soluble compounds. In turn, RMEs on the cellulose fraction were positively related to dissimilarity in cellulose concentrations among co-decomposing litter types. Such increasing dissimilarity in cellulose concentrations could bolster the decomposition of the available cellulose as a readily available source of carbon and energy for decomposers. This is in line with findings by Talbot and Treseder (2012) who reported higher cellulose decomposition in litter of *Arabidopsis thaliana* with low cellulose content, which they attributed to the mining of cellulose by decomposers. In turn, the RMEs on the lignin fraction turned from antagonistic to null as the dissimilarity in N and lignin concentrations increased among co-decomposing litter types. While mechanisms underlying antagonistic effects and their relation to litter dissimilarity are unclear, the reduction of their strength could be due to synergistic effects driven by increasing N and lignin concentration dissimilarity (Cuchietti et al., 2014) cancelling out the antagonistic effects. Regardless of the underlying mechanisms, our results indicate that one reason for the lack of emerging litter dissimilarity index as a common driver of litter diversity effects may be due to the focus on bulk litter decomposition (Kou et al., 2020), which blurs the contrasted responses of different litter fractions to litter diversity. Disentangling the responses of distinct litter fractions thus appears as a way forward to understanding the drivers of litter diversity effect in future studies.

Another important finding of our study is that, in contrast with our fourth hypothesis, the magnitude of the RMEs on the mass loss of the different litter fractions consistently decreased throughout decomposition, leading to non-significant litter diversity effects on the decomposition of all fractions after 3.5 year of decomposition, once the litter had lost more than 60% of its mass. This general fading of litter diversity effects contrasts with previous reports of more pronounced litter diversity effects at later decomposition stages (Wu et al., 2013; Kou et al., 2020), but is in line with another study (Butenschoen et al., 2014). One possible explanation is that as litter physicochemical characteristics converge during decomposition (Moore et al., 2011), dissimilarity-driven RMEs decrease. Overall, this clear fading of litter diversity effects suggests that they may only be transient and affect more the decomposition dynamic than the proportion of undecomposed litter in the long-term. Interestingly, as a consequence of synergistic and antagonistic effects on the different fractions decreasing with time, the magnitude of RMEs on bulk litter decomposition remained relatively constant throughout decomposition. This suggests that the contrasting dynamics of the RMEs on different fractions may remain unnoticed when the fate of litter fractions is not tracked throughout decomposition.

Collectively, our finding that litter diversity has contrasting effects depending on the litter fractions considered, with positive effect of mixing for labile fraction and negative effects for recalcitrant fractions, presents a promising research avenue to understand and predict the role of litter diversity on decomposition. The fading of synergistic and antagonistic litter diversity effects throughout decomposition also clearly shows the dynamic of such effects and highlights the need to focus on long-term decomposition. It is important to note that plant diversity not only affects decomposition directly through altered litter diversity but also indirectly through changes in microenvironmental conditions (Joly et al., 2017) – an aspect that was not considered in our study and that should be considered to evaluate the overall effect of plant diversity on biogeochemical cycles. Further research on the response of litter fraction decomposition to plant diversity is thus needed and may help making sense of the largely idiosyncratic responses of decomposition to litter diversity reported to date.

Acknowledgements

This work was funded by the Sichuan Provincial Science and Technology Development Special Program for Central Government Guidance (2020ZYD049), the National Natural Science Foundation of China (31570605), and the Foundation of Key Laboratory of Southwest China Wildlife Resources Conservation, China West Normal University, Ministry of Education, Nanchong, 637009, P. R. China (XNYB21-02).

References

Barantal, S., Schimann, H., Fromin, N., Hättenschwiler, S., 2014. C , N and P fertilization in an Amazonian rainforest supports stoichiometric dissimilarity as a driver of litter diversity effects on decomposition. Proceedings of the Royal Society of London B: Biological Sciences 281, 20141682. doi:10.1098/rspb.2014.1682

Berg, B., McClaugherty, C., 2020. Plant Litter Decomposition, Humus Formation, Carbon Sequestration., Fourth Edition. Springer-Verlag, Heidelberg, Germany.

Butenschoen, O., Krashevska, V., Maraun, M., Marian, F., Sandmann, D., Scheu, S., 2014. Litter mixture effects on decomposition in tropical montane rainforests vary strongly with time and turn negative at later stages of decay. Soil Biology and Biochemistry 77, 121–128. doi:10.1016/j.soilbio.2014.06.019

Chapman, S.K., Koch, G.W., 2007. What type of diversity yields synergy during mixed litter decomposition in a natural forest ecosystem? Plant and Soil 299, 153–162. doi:10.1007/s11104-007-9372-8

Chen, Y., Ma, S., Jiang, H., Yangzom, D., Cheng, G., Lu, X., 2021. Decomposition time, chemical traits and climatic factors determine litter-mixing effects on decomposition in an alpine steppe ecosystem in Northern Tibet. Plant and Soil 459, 23–35. doi:10.1007/s11104-019-04131-9

Cuchietti, A., Marcotti, E., Gurvich, D.E., Cingolani, A.M., Pérez Harguindeguy, N., 2014. Leaf litter mixtures and neighbour effects: Low-nitrogen and high-lignin species increase decomposition rate of high-nitrogen and low-lignin neighbours. Applied Soil Ecology 82, 44–51. doi:10.1016/j.apsoil.2014.05.004

Gartner, T.B., Cardon, Z.G., 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104, 230–246. doi:10.1111/j.0030-1299.2004.12738.x

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H., Hättenschwiler, S., 2010. Diversity meets decomposition. Trends in Ecology and Evolution 25, 372–380. doi:10.1016/j.tree.2010.01.010

Grossman, J.J., Cavender-Bares, J., Hobbie, S.E., 2020. Functional diversity of leaf litter mixtures slows decomposition of labile but not recalcitrant carbon over two years. Ecological Monographs 90, 1–19. doi:10.1002/ecm.1407

Handa, I.T., Aerts, R., Berendse, F., Berg, M.P., Bruder, A., Butenschoen, O., Chauvet, E., Gessner, M.O., Jabiol, J., Makkonen, M., McKie, B.G., Malmqvist, B., Peeters, E.T.H.M., Scheu, S., Schmid, B., Van Ruijven, J., Vos, V.C.A., Hättenschwiler, S., 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature 509, 218–221. doi:10.1038/nature13247

Hättenschwiler, S., Tiunov, A. V., Scheu, S., 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annual Review of Ecology, Evolution, and Systematics 36, 191–218. doi:10.1146/annurev.ecolsys.36.112904.151932

Joly, F.X., Milcu, A., Scherer-Lorenzen, M., Jean, L.K., Bussotti, F., Dawud, S.M., Müller, S., Pollastrini, M., Raulund-Rasmussen, K., Vesterdal, L., Hättenschwiler, S., 2017. Tree species diversity affects decomposition through modified micro-environmental conditions across European forests. New Phytologist 214, 1281–1293. doi:10.1111/nph.14452

Jonsson, M., Wardle, D.A., 2008. Context dependency of litter-mixing effects on decomposition and nutrient release across a long-term chronosequence. Oikos 117, 1674–1682. doi:10.1111/j.1600-0706.2008.16810.x

Kou, L., Jiang, L., Hättenschwiler, S., Zhang, M., Niu, S., Fu, X., Dai, X., Yan, H., Li, S., Wang, H., 2020. Diversity-decomposition relationships in forests worldwide. ELife 9, 1–51. doi:10.7554/eLife.55813

Kuebbing, S.E., Bradford, M.A., 2019. The potential for mass ratio and trait divergence effects to explain idiosyncratic impacts of non-native invasive plants on carbon mineralization of decomposing leaf litter. Functional Ecology 33, 1156–1171. doi:10.1111/1365-2435.13316

Makkonen, M., Berg, M.P., van Logtestijn, R.S.P., van Hal, J.R., Aerts, R., 2013. Do physical plant litter traits explain non-additivity in litter mixtures? A test of the improved microenvironmental conditions theory. Oikos 122, 987–997. doi:10.1111/j.1600-0706.2012.20750.x

Moore, T.R., Trofymow, J.A., Prescott, C.E., Titus, B.D., 2011. Nature and nurture in the dynamics of C, N and P during litter decomposition in Canadian forests. Plant and Soil 339, 163–175. doi:10.1007/s11104-010-0563-3

Schimel, J.P., Cates, R.G., Ruess, R., 1998. The role of balsam poplar secondary chemicals in controlling soil nutrient dynamics through succession in the Alaskan taiga. Biogeochemistry 42, 221–234.

Schimel, J.P., Hättenschwiler, S., 2007. Nitrogen transfer between decomposing leaves of different N status. Soil Biology and Biochemistry 39, 1428–1436. doi:10.1016/j.soilbio.2006.12.037

Shipley, B., Tardif, A., 2021. Causal hypotheses accounting for correlations between decomposition rates of different mass fractions of leaf litter. Ecology 102, 1–9. doi:10.1002/ecy.3196

Talbot, J.M., Treseder, K.K., 2012. Interactions among lignin, cellulose, and nitrogen drive litter chemistry-decay relationships. Ecology 93, 345–354. doi:10.1890/11-0843.1

Tardif, A., Shipley, B., 2015. The relationship between functional dispersion of mixed-species leaf litter mixtures and species’ interactions during decomposition. Oikos 124, 1050–1057. doi:10.1111/oik.01686

Vos, V.C.A., van Ruijven, J., Berg, M.P., Peeters, E.T.H.M., Berendse, F., 2013. Leaf litter quality drives litter mixing effects through complementary resource use among detritivores.

Oecologia 173, 269–280. doi:10.1007/s00442-012-2588-1

Wang, L, Chen, Y., Zhou, Y., Xu, Z., Tan, B., You, C., Zhang, L., Li, H., Zheng, H., Guo, L., Wang, L, Huang, Y., Zhang, J., Liu, Y., 2021a. Environmental conditions and litter nutrients are key determinants of soluble C, N, and P release during litter mixture decomposition. Soil and Tillage Research 209, 104928. doi:10.1016/j.still.2020.104928

Wang, L, Chen, Y., Zhou, Y., Zheng, H., Xu, Z., Tan, B., You, C., Zhang, L., Li, H., Guo, L., Wang, L, Huang, Y., Zhang, J., Liu, Y., 2021b. Litter chemical traits strongly drove the carbon fractions loss during decomposition across an alpine treeline ecotone. Science of the Total Environment 753, 142287. doi:10.1016/j.scitotenv.2020.142287

Wardle, D.A., Bonner, K.I., Nicholson, K.S., 1997. Biodiversity and plant litter: experimental evidence which does not support the view that enhanced species richness improves ecosystem function. Oikos 79, 247–258. doi:https://doi.org/10.2307/3546010

Wu, D., Li, T., Wan, S., 2013. Time and litter species composition affect litter-mixing effects on decomposition rates. Plant and Soil 371, 355–366. doi:10.1007/s11104-013-1697-x

Figures

Figure 1: Relative mixing effects (RMEs) on (a) bulk litter mass loss, (b) soluble mass loss, (c) cellulose mass loss and (d) lignin mass loss, as a function of total litter mass loss. Black lines represent the regression lines between RMEs and litter mass loss, with grey areas representing the 95% confidence intervals of regression lines. For each fraction, inserts represent the RMEs on *k* constants for each litter mixture. All FT: Litter mixture with all six functional types; -EC: Litter mixture without evergreen conifers; -ES: Litter mixture without evergreen shrubs; -FE: Litter mixture without ferns; -GR: Litter mixture without graminoids; -DS: Litter mixture without deciduous shrubs litter loss; -FO: Litter mixture without forbs.

Figure 2: Principal component analysis (PCA) of litter functional diversity. Black lines depict the variable loadings, and the colored lines depict the correlation between the PCA axes and the RMEs of litter C fractions (*k* constant) (green), and RMEs of bulk litter mass (*k* constant) (yellow).

Figure 3: Relative mixing effects (RMEs) on (a) bulk litter mass loss (*k* constant), (b) soluble mass loss (*k* constant), (c) cellulose mass loss (*k* constant), and (c) lignin mass loss (*k* constant) as a function of litter functional diversity PC1, PC3, PC2, and PC1 scores (fixed predictor), respectively. Plot nested within transect is included as a random predictor.

Figure 1

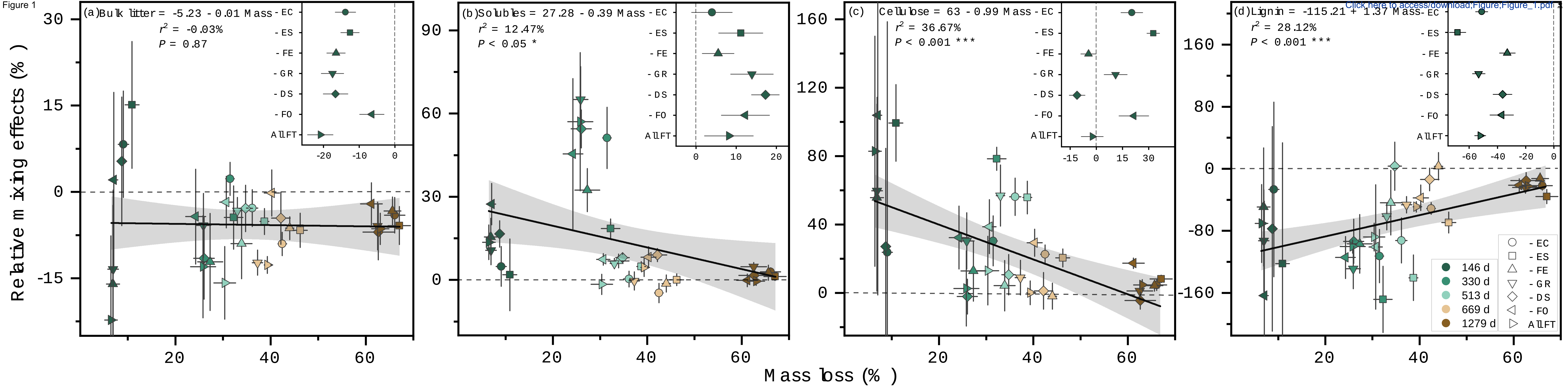

(a) Bulk litter = -5.23 - 0.01 Mass
r^2 = -0.03%
P = 0.87
(b) Solubles = 27.28 - 0.39 Mass
r^2 = 12.47%
P < 0.05 *
(c) Cellulose = 63 - 0.99 Mass
r^2 = 36.67%
P < 0.001 ***
(d) Lignin = -115.21 + 1.37 Mass
r^2 = 28.12%
P < 0.001 ***
Relative mixing effects (%)
Mass loss (%)
30
15
0
-15
90
60
30
0
160
120
80
40
0
160
80
0
-80
-160
20
40
60
EC
ES
FE
GR
DS
FO
All FT
-20
-10
0
0
10
20
-15
0
15
30
-60
-40
-20
0
○ EC
□ ES
△ FE
▽ GR
◇ DS
◁ FO
▷ All FT
● 146 d
● 330 d
● 513 d
● 669 d
● 1279 d

Figure 2

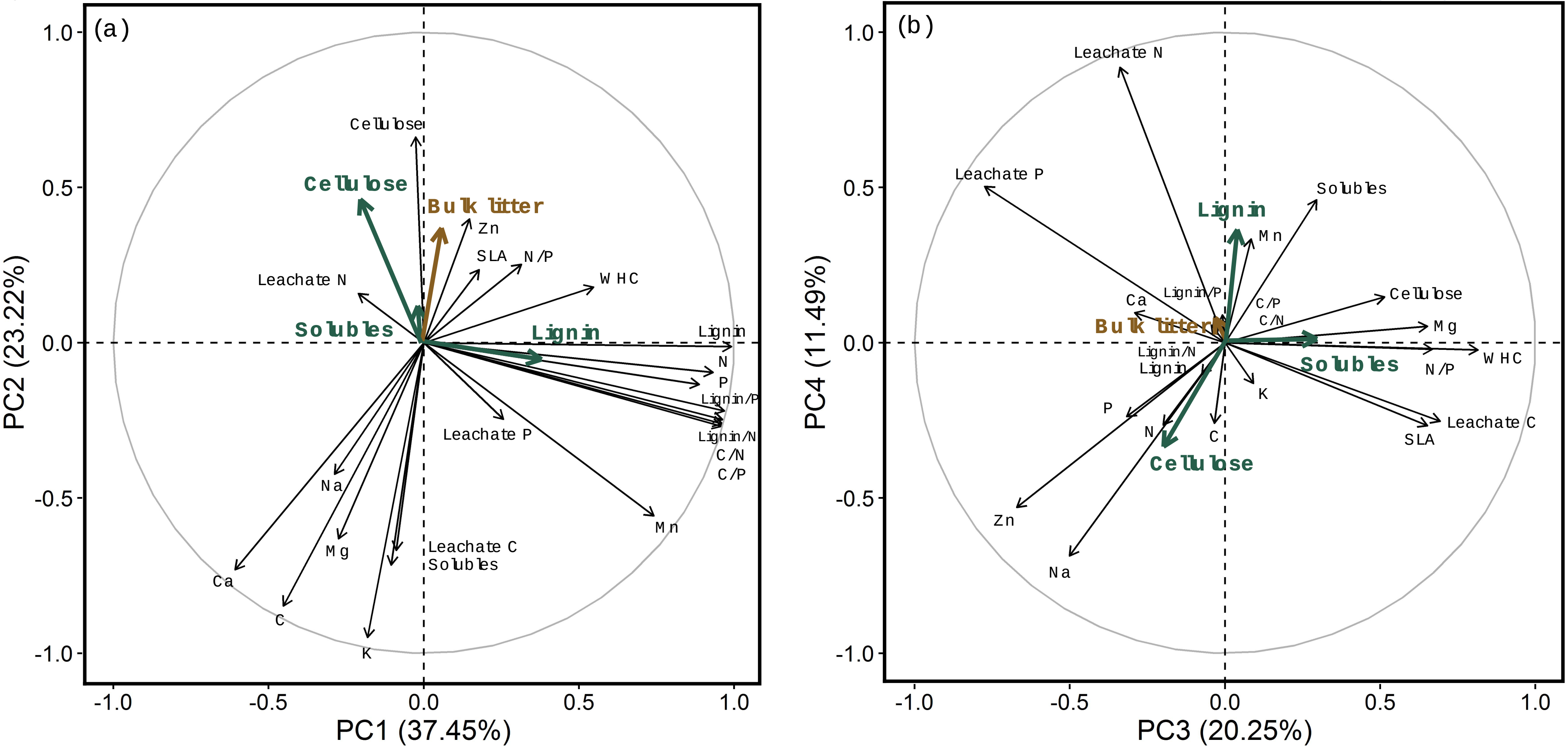
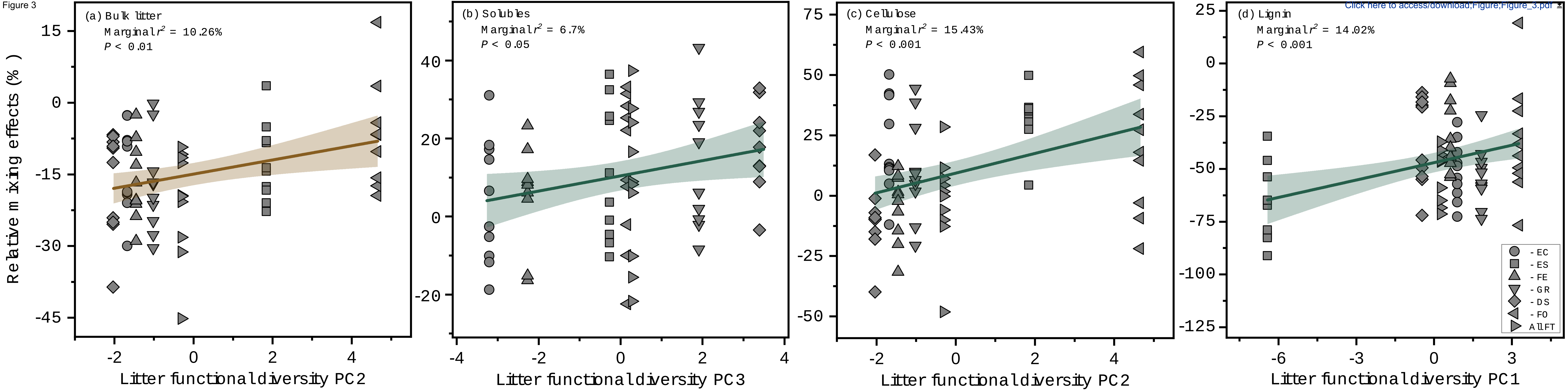

(a)
PC2 (23.22%)
PC1 (37.45%)
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
Cellulose
Cellulose
Bulk litter
Zn
SLA
N/P
Leachate N
WHC
Solubles
Lignin
Lignin
N
P
Lignin/P
Leachate P
Lignin/N
C/N
C/P
Na
Mg
Ca
C
K
Leachate C
Solubles
Mn
(b)
PC4 (11.49%)
PC3 (20.25%)
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
Leachate N
Leachate P
Solubles
Lignin
Mn
Lignin/P
Ca
C/P
C/N
Cellulose
Mg
Bulk litter
Lignin/N
Lignin
Solubles
N/P
WHC
K
P
N
C
Leachate C
SLA
Cellulose
Zn
Na

Figure 3


(a) Bulk litter
Marginal r^2 = 10.26%
P < 0.01
Relative mixing effects (%)
15
0
-15
-30
-45
-2
0
2
4
Litter functional diversity PC2
(b) Solubles
Marginal r^2 = 6.7%
P < 0.05
40
20
0
-20
-4
-2
0
2
4
Litter functional diversity PC3
(c) Cellulose
Marginal r^2 = 15.43%
P < 0.001
75
50
25
0
-25
-50
-2
0
2
4
Litter functional diversity PC2
(d) Lignin
Marginal r^2 = 14.02%
P < 0.001
25
0
-25
-50
-75
-100
-125
-6
-3
0
3
Litter functional diversity PC1
● - EC
■ - ES
▲ - FE
▼ - GR
◆ - DS
◀ - FO
▶ All FT

Supplementary Material for online publication only