Plant diversity increases soil microbial activity and soil carbon storage

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Plant diversity strongly influences ecosystem functions and services, such as soil carbon storage. However, the mechanisms underlying the positive plant diversity effects on soil carbon storage are poorly understood. We explored this relationship using long-term data from a grassland biodiversity experiment (The Jena Experiment) and radiocarbon (14C) modelling. Here we show that higher plant diversity increases rhizosphere carbon inputs into the microbial community resulting in both increased microbial activity and carbon storage. Increases in soil carbon were related to the enhanced accumulation of recently fixed carbon in high-diversity plots, while plant diversity had less pronounced effects on the decomposition rate of existing carbon. The present study shows that elevated carbon storage at high plant diversity is a direct function of the soil microbial community, indicating that the increase in carbon storage is mainly limited by the integration of new carbon into soil and less by the decomposition of existing soil carbon.

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Climate and land use change can cause alterations in biodiversity with significant consequences for ecosystem functions and services. Quantifying the impact of biodiversity loss on ecosystem services requires long-term investigations in replicated experimental systems. This is especially true for soil carbon storage as changes in stocks are slow, the spatial heterogeneity is large, and the involved processes are complex. Soils store the vast majority of terrestrial carbon, and increased soil carbon storage may counteract the increase in atmospheric carbon dioxide. Traditional explanatory theories focus on the recalcitrance of less-reactive compounds and physical protection as controls of soil carbon stocks, while more recent theories highlight the importance of soil microorganisms for the persistence of soil organic matter. With respect to microbial regulation, emerging theories focus on either spatial factors, which constrain the accessibility of substrates to decomposer organisms; or microbial carbon re-cycling and the consequent microbial production of soil organic matter through microbially derived products. Alternatively or in parallel, higher activity are the main drivers explaining the positive effect of plant species richness on soil carbon storage, showing that fine root carbon inputs and microbial activity are the main drivers explaining the positive effect of plant diversity on soil carbon storage. Low root biomass on plots with legumes may have caused the negative effect of legumes on microbial activity via reduced carbon inputs. Importantly, the increased soil carbon storage was only directly related to higher microbial activity. This potential chain of causality was the best model that adequately explained the data (Supplementary Table 2; Supplementary Notes 1–4), also when soil carbon sequestration was set up in a path model as a time series (Supplementary Fig. 2), which increased the likelihood of the model.

**Mechanisms of soil organic carbon storage.** In this strictly confirmatory approach, we tested different hypotheses (Table 2; Fig. 2), resulting in a set of different path models (Supplementary Fig. 1), assessing whether the models deviate or not from the observed data structure. All individual models were tested for their adequacy in reflecting the observed data. Path analyses revealed that plant species richness had direct positive effects on root carbon inputs as well as metabolic activity of soil microorganisms, measured as basal respiration (Fig. 3a,b; Methods). There was no direct relationship between plant species richness or the presence of legumes and soil carbon storage, showing that fine root carbon inputs and microbial activity are the main drivers explaining the positive effect of plant diversity on soil carbon storage. Low root biomass on plots with legumes may have caused the negative effect of legumes on microbial activity via reduced carbon inputs. Importantly, the increased soil carbon storage was only directly related to higher microbial activity. This potential chain of causality was the best model that adequately explained the data (Supplementary Table 2; Supplementary Notes 1–4), also when soil carbon sequestration was set up in a path model as a time series (Supplementary Fig. 2), which increased the likelihood of the model.

**Dynamics of soil organic carbon.** Radiocarbon-based modelling of SOM storage (Methods) suggested that the increased carbon storage in plots with higher plant species richness was mainly due to the accumulation of ‘new’ carbon (Fig. 4a). Furthermore, the sequestration of ‘new’ carbon and the loss of existing ‘old’ carbon across all diversity treatments had increased by 27% to 4.74 ± 0.28 g kg⁻¹ (mean ± s.e.m.). An increase in SOM was expected because the experiment had been established on a ploughed arable field turned into permanent grassland. The increase in soil carbon concentration was highly correlated with sown plant species richness (P < 0.001, Fig. 1). The presence of legumes negatively affected soil carbon concentration (P = 0.040, Fig. 1), while other plant functional groups or their richness did not significantly influence carbon concentration (Table 1; Supplementary Table 1).

**Results**

**Effects of plant diversity on soil organic carbon changes.** In 2011, 9 years after the establishment of The Jena Experiment, soil carbon concentration in the topsoil layer (0–5 cm, averaged between explanatory variables affecting soil carbon storage. Furthermore, to determine whether soil carbon accumulation resulted from increased root-derived carbon inputs or reduced decomposition, we modelled the carbon flow in the system using observations of soil carbon stocks and radiocarbon (¹⁴C) in soil organic matter (SOM) as a double constraint. The combined results of path analysis and soil carbon modelling suggest a unique cause for enhanced carbon storage with plant diversity in our experimental plots. Although both approaches are based on data from the top, biologically most active soil layer (0–5 cm), we were able to generalize the proposed mechanism of plant diversity effects on soil carbon storage to deeper soil layers (0–30 cm).
These opposite patterns are the transfer or processing rate stored in the slow-cycling carbon (Fig. 4d). An explanation for reduced at higher plant diversity, and more ‘new’ carbon was in the more sustainable slow-cycling carbon these losses were average only 9.6% of ‘old’ carbon. Interestingly, carbon losses 2 and 4). At the same time, high-diversity plots primed on more carbon compared with low-diversity plots (richness levels 1, 2 and 4). The presence of legumes increases microbial activity14,42-44. In fact, bacterial and fungal diversity increased with higher plant species richness in The Jena Experiment (Table 1; Fig. 6). And finally, a shift in the metabolic activity of soil microorganisms towards anabolic activity with plant diversity resulting in increased necromass accumulation over time23, indicated by slightly but significantly more microbial carbon per unit root carbon in high-diversity plots ($R^2 = 0.06, P = 0.025)$.

The missing direct path from legumes to microbial activity indicates minor importance of improvement of soil nitrogen status (measured as changes in $^{15}$N of bulk soil, Table 1) for soil microbial activity, and thus for carbon storage. This was partly unexpected as legumes have been reported to increase the availability of nitrogen in soils35,36 and are therefore suggested to increase plant biomass production (above and below ground)24, resulting in increased metabolic activity of soil microorganisms37.

Table 1 | Results of ANOVAs on the effects of the experimental variables.

| Pathway | Hypothesized mechanism |
| --- | --- |
| 1 Plant species richness → carbon inputs | Plant diversity increases root biomass or root carbon concentration24,29,39 |
| 2 Plant species richness → microbial activity | Plant diversity increases microbial community activity20,30 |
| 3 Legumes → carbon inputs | Plant community composition, such as the presence of legumes, influences the amount of root carbon residues24 |
| 4 Legumes → microbial activity | The presence of legumes decreases soil microbial activity24 |
| 5 Carbon inputs → microbial activity | Positive effects of root carbon residues on soil microbial activity20,37 |
| 6 Carbon inputs → carbon storage | Carbon storage increases with higher inputs of root carbon residues or with more plant-derived recalcitrant compounds70 |
| 7 Microbial activity → carbon storage | Microbial community activity influences soil carbon storage42,44 |
| 8 Carbon storage → microbial activity | Higher soil organic carbon content increases soil microbial community activity22 |
| 9 Legumes → carbon storage | The presence of legumes influences carbon storage by other mechanisms than through root carbon inputs and microbial activity |
| 10 Plant species richness → carbon storage | Plant species richness influences carbon storage by other mechanisms than root carbon inputs and microbial activity |

Impact of plant diversity (plant species richness (PSR, log transformed) and number of plant functional groups (FGs)) and the presence of distinct plant functional groups (legumes, grasses, small herbs, tall herbs) on changes in soil organic carbon (Acc$_{0-5}$ and 0-30 cm), genetic diversity (determined using terminal-restriction fragment length polymorphism (TRFLP)) of the soil microbial community (fungi and bacteria) and changes in $^{15}$N of bulk soil, Table 1) for soil microbial activity, and thus for carbon storage. This was partly unexpected as legumes have been reported to increase the availability of nitrogen in soils35,36 and are therefore suggested to increase plant biomass production (above and below ground)24, resulting in increased metabolic activity of soil microorganisms37.

Table 2 | Potential relations between the considered variables.

| Pathway | Hypothesized mechanism |
| --- | --- |
| 1 Plant species richness → carbon inputs | Plant diversity increases root biomass or root carbon concentration24,29,39 |
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Hypothesized mechanisms of the individual paths in the conceptual model (Fig. 2). Arrows indicate the direction of the paths.

Discussion

The results reported in this study indicate that storage of soil carbon is governed by the metabolic activity of soil microbes, which is mediated by plant diversity via higher root inputs and other yet unidentified mechanisms, as suggested by the direct relation in the path model (Fig. 3a). A number of processes influence microbial activity: First, a denser vegetation in highly diverse plant communities reduces evaporation from the topsoil, which in turn promotes higher soil microbial activity and growth30. Second, soil carbon storage is linked to root inputs31,32 including root exudation, which is known to change the activity and composition of the microbial community33. Indeed, carbon uptake in rhizospheric microorganisms at high plant diversity was increased compared with low plant diversity (Fig. 5) as indicated by a complementary $^{13}$CO$_2$ labelling experiment in the Ecotron facility in Montpellier (Methods). This showed higher microbial label uptake was most likely attributed to higher levels of root exudation with increasing plant diversity, as the soil microbial community was sampled immediately after a 3-week label application and the label enrichment in roots and root sugars was not affected by diversity (data not shown). Third, increasing diversity of soil microbial communities increases microbial respiration34. In fact, bacterial and fungal diversity increased with higher plant species richness in The Jena Experiment (Table 1; Fig. 6). And finally, a shift in the metabolic activity of soil microorganisms towards anabolic activity with plant diversity resulting in increased necromass accumulation over time23, indicated by slightly but significantly more microbial carbon per unit root carbon in high-diversity plots ($R^2 = 0.06, P = 0.025$).
However, the presence of legumes did not increase root biomass production and the metabolic activity of soil microorganisms in The Jena Experiment\textsuperscript{20,38}. Although the increase in total nitrogen content was highly correlated with the increase in soil organic carbon ($R^2 = 0.79, P < 0.001$), the lower root biomass on legume plots\textsuperscript{29} likely caused the negative legume effect on soil carbon storage. However, the legume effect might be most pronounced in the topsoil as it was not significant when considering the top 30 cm of the soil (Table 1). In contrast, the positive plant diversity effect on soil carbon storage (Table 1), root biomass\textsuperscript{39} and soil microbial biomass\textsuperscript{40} was also found for deeper soil layers in The Jena Experiment. Despite the consistency of the plant diversity effect, it may be strongest in the topsoil and decrease with soil depth\textsuperscript{25,39}, which may be due to the fact that root carbon inputs\textsuperscript{24,39} and microbial activity\textsuperscript{41} decrease with soil depth. Therefore, the mechanism proposed for the topsoil is very likely to also be relevant for deeper soil layers, but the decreased biological activity at the deeper soil layers has to be taken into account. Moreover, other mechanisms such as spatial separation from decomposers\textsuperscript{9,12} might become more important with soil depth.

The positive plant diversity effect on carbon storage is driven by root carbon inputs, but mediated by the soil microbial community. Longer term, we expect this link to become even more important through both an increase of the positive plant diversity effect on plant (root) biomass production\textsuperscript{6,39} and an increased microbial contribution to soil carbon storage by elevated soil microbial activity\textsuperscript{20}. Both mechanisms in concert might be responsible for an increase of the plant diversity effect on carbon storage, as indicated by the time series path model (Supplementary Fig. 2).

In summary, higher levels of carbon inputs to the soil and more favourable microclimatic conditions linked to more diverse plant communities result in more active, more abundant and more diverse soil microbial communities. Microbial activity increases the turnover rates of root litter and exudates as indicated by increased microbial respiration. Thus, microbial products associated with increased microbial respiration such as microbial necromass end up in slow-cycling SOM pools in the form of reduced organic material\textsuperscript{10,14}. The importance of microbial residues for soil carbon storage is suggested to even increase over time\textsuperscript{23}. Thus, in line with the recent debate\textsuperscript{42–44}, our findings challenge previous views underestimating or even negating the influence of size and activity of soil microorganisms on soil carbon storage.

Our data from long-term field experiments emphasize the importance of plant diversity and its effects on soil microorganisms for ecosystem carbon storage. Although we cannot rule out that the proposed mechanisms are particularly pronounced at the field site of The Jena Experiment, the underlying relationships between plant diversity and soil carbon storage\textsuperscript{17,24}, soil microbial biomass and activity\textsuperscript{37,45,46}, and root biomass\textsuperscript{24,47} have been reported in several independent experimental settings providing some evidence that the proposed links are of general significance. This leads us to reconsider the role of soil microorganisms as sources rather than sinks for slow-cycling organic matter. Thus, our results suggest that the activity and composition of soil microbial communities can serve as a proxy for carbon transfer into sustainable slow-cycling forms of soil carbon, and that plant diversity and associated soil microbial communities can significantly contribute to sequestration of atmospheric carbon dioxide.
Methods

Study site of The Jena Experiment. Our study was carried out as a part of The Jena Experiment, a large-scale grassland diversity experiment. The experiment is located on the floodplain of the Saale River near the city of Jena (Thuringia, Germany; 50°17'50"N, 11°35'0"E). The soil of the field site is classified as Eutric Fluvisol. In spring 2002, 82 experimental grassland plots of 20 × 20 m were established. One monoculture plot was abandoned due to poor establishment, making the total number of plots 81 instead of the 82 originally planned. Plots are arranged in four blocks to account for changes in abiotic factors with distance from the river. Soil texture in the upper soil ranges from sandy loam to silty clay with increasing distance from the river. Sand content declines from 40% near the river to 7% at the furthest plot, while silt content increases from 44 to 69%, respectively. The clay content ranges from 16 to 24%, but is not related to distance from the river. For the 40 years before establishing the experiment, the field site was a cultivated field with mineral fertilizer input. The initial physico-chemical
properties, such as pH (7.1–8.4), soil organic carbon (5–33 g C kg−1), and soil nitrogen concentrations (1.0–2.7 g N kg−1), varied across the field site and are considered to be representative of the block design of The Jena Experiment.56

The plots differed in the number of plant species (1, 2, 4, 8, 16 and 60 species), randomly chosen from a common pool of 60 plant species, and the number of plant functional groups, that is, grasses, legumes, small herbs and tall herbs, which were classified into four functional groups based on morphological, phenological and physiological traits; for details see ref. 26. Experimental communities are weeded manually twice a year to maintain the levels of diversity and mown twice yearly in June and September as is typical for hay meadows in Central Europe.

Sampling and laboratory analyses. Data included in this paper originated from soil samples taken at a depth of 0–5 cm, where biological activity is at a maximum. Fine root biomass in some cases had to be extrapolated to obtain estimates for the topsoil (0–5 cm), as not all sampling campaigns were set up for stratified sampling (see below).

Soil organic carbon. Soil sampling was performed before sowing in April 2002 and was repeated in April in the years 2004, 2006, 2008 and 2011, as described in more detail in ref. 25. In short, three soil samples were taken per plot (4.8 cm in diameter, 0–30 cm deep) using a split tube sampler (Eijkelkamp Agrisearch Equipment, Giesbeek, the Netherlands). In the year 2002, a total of five soil samples were taken per plot. The soil was dried, sieved (2 mm mesh) and milled (4 min, frequency 30%). Total carbon of ground samples was determined by an elemental analyser after combustion at 1,150 °C (Elementar Analysensysteme GmbH, Hanau, Germany). Inorganic carbon concentration was measured by elemental analysis after removing organic carbon for 16 h by gel filtration using Sephadex G50 (Sigma-Aldrich, Gillingham, UK) before digestion with restriction endonucleases MspI (CCGG) and TaqI (TGGCA; Promega, Southampton, UK) for bacteria and fungi, respectively. A 3730 DNA analyser (Applied Biosystems, CA, USA) was used for fragment analysis, before binning of individual terminal-restriction fragments using Genemarker software (SoftGenetics, PA, USA). The relative abundance of constituent restriction fragments was calculated by dividing the intensity of each fragment by the total intensity of all fragments before calculation of Simpson’s diversity index.

Statistical analyses of main effects of plant diversity. The Jena Experiment is based on a factorial design with different combinations of sown plant species richness and number of functional groups. To assess the design variables, analyses of variance with sequential sum of squares (type I SS) were applied using R60. The ‘block effect’, mainly accounting for differences in soil texture among the blocks26, was included as random factor and fitted first, followed by plant species richness and functional groups. Finally, the presence of each plant functional group (legumes, grasses, small herbs and tall herbs) was included into the model in a series of alternative models.

Path analysis. We applied path analyses (maximum likelihood)28 to explore the potential causal relationships between plant diversity and community composition and soil carbon storage using the lavaan package in R61. Depending on the results of the analysis of variance approach, all variables with significant effect on changes of soil carbon content were included as exogenous (predictor) variables. As variables potentially mediating plant community effects we included fine root carbon as measure of carbon input11,12 and basal respiration as measure of microbial metabolic activity20,34,37. We tested four different ways how plant diversity can affect carbon storage: due to carbon input from roots, due to microbial activity, due to both root inputs as well as microbial activity, or directly via plant diversity, indicating that other mechanisms than root carbon input and microbial activity underlie the plant diversity effect. Each of the four storage ways comprised a set of models (Supplementary Fig. 1), differing in the relations from plant diversity to basal respiration and fine root carbon to basal respiration. For all models, the adequacy of the hypothesized structural relationships and the data was verified using χ²-tests and the residual mean square error of approximation. To test whether carbon storage is a function of the microbial activity or vice versa, we tested all models twice, first with the path from basal respiration towards soil carbon content, as ‘sink models’ (as described above, Supplementary Note 1) and second with a path from soil carbon content towards basal respiration as ‘source models’ (Supplementary Note 2). In the final step, we identified the minimal adequate (most parsimonious) model based on the Bayses Information Criterion. Therefore, all adequate models were compared (Supplementary Note 3), including nonsignificant pathways and when only significant pathways are contained.

Eisenhauer et al.27 and Ravenek et al.29 showed that plant species richness was not closely linked to basal respiration and root standing biomass in the first years of the experiment; therefore, we did not use data from the first 2 years in the present analyses. Missing values were replaced by the mean. All variables were standardized to (0, 1). The total sample size was N = 81.

Three-pool 13C model with serial structure. To describe soil organic carbon dynamics in The Jena Experiment, we implemented a dynamic model of the form

\[
\frac{\text{d}C_t}{\text{d}t} = -k_C C_t
\]

where \(C_t\), \(C_s\) and \(C_p\) represent carbon storage in the fast, slow and passive pool, respectively. \(I\) represents the total amount of carbon input to the first 5 cm of the soil, and \(k_f\), \(k_s\) and \(k_p\) represent first-order decomposition rates for the fast, slow and passive pools, respectively. The transfer coefficients \(z_{f-p}\) and \(z_{s-p}\) represent the proportion of the decomposed carbon from each pool that is transferred to a slower pool.

One particular aspect of this model, suggested from the path analysis, is that we represent the effects of the microbial community on decomposition as a control on
the transfer rate. That is, we assume

\[ x_{2t_k} = f \cdot MB, \]  

where MB is the microbial biomass and \( f \) is a scaling factor. Notice that we do not represent the microbial biomass as an additional pool in our dynamical system because it leads to unrealistic nonlinear behaviour

To account for radiocarbon dynamics, we implemented a model similar to equation (1) representing each pool as a fraction of radiocarbon with respect to an internationally agreed standard. The model has similar form as equation (1) with an additional term accounting for radioactive decay. The model was implemented in the R environment for computing with the SoilR package. The passive pool is mainly needed to account for the low radiocarbon values in the bulk soil organic matter. As described by other authors, such low radiocarbon values are common for arable fields in Europe.

On the basis of our findings in the path analysis, we implemented a measure of soil microbial community activity (basal respiration) as a proxy for the transfer between the fast and slow carbon pool. Unfortunately, no data on basal respiration were available for arable control plots, which would have been needed for the spin-up (please see section ‘Spin up and parameter estimation for the spin up’). Thus, we took advantage of the microbial biomass (based on the chloroform fumigation extraction analysis) instead. Basal respiration and microbial biomass were highly correlated (Fig. 3c). For each level of plant species richness, we applied a model with the level specific parameter estimation (Table 1; Fig. 3b). However, we could neither evaluate the real carbon input (carbon of standing fine root biomass and root exudates, root tips and so on) nor the extent of the transfer coefficient. Thus, we applied a set of different models for each level of plant diversity, modified in the carbon input and transfer coefficient. Implementing the transfer coefficient between the fast and slow pool depending on soil microbial biomass resulted in good agreement between model predictions and measured data (Table 3; Supplementary Table 3; and Supplementary Fig. 3).

Good agreement between model predictions and measured data (Table 3; Fig. 3b). However, we could neither evaluate the real carbon input (carbon of standing fine root biomass and root exudates, root tips and so on) nor the extent of the transfer coefficient. Thus, we applied a set of different models for each level of plant diversity, modified in the carbon input and transfer coefficient. Implementing the transfer coefficient between the fast and slow pool depending on soil microbial biomass resulted in good agreement between model predictions and measured data (Table 3; Supplementary Table 3; and Supplementary Fig. 3).

We calculated new carbon accumulated and loss of old carbon: on the basis of the mass balance and the isotopic mass balance for the isotopic mass ratio (equation (3)) and the yearly (\( t \)) amount of new carbon sequestered (\( C_{new} \)) and loss of the old carbon (\( C_{old} \)).

\[ \Delta C_{new} = C_{new} - C_{old}; \]
\[ \Delta C_{old} = C_{old} - C_{new}; \]
\[ \delta_{\Delta C} = \Delta C_{new} - \Delta C_{old} = \delta_{\Delta C} = \delta_{\Delta C_{new}} - \delta_{\Delta C_{old}} = \delta_{\Delta C_{new}} - \delta_{\Delta C_{old}} = \delta_{\Delta C_{new}} + \delta_{\Delta C_{old}}. \]

Spin up and parameter estimation for the spin up. The objective of the spin up simulations is to match the carbon stocks and radiocarbon data for the pre-experimental conditions. We let the spin up simulations run for 10,000 years to assure the steady state conditions. We let the spin up simulations run for 10,000 years to assure the steady state conditions. We let the spin up simulations run for 10,000 years to assure the steady state conditions. We let the spin up simulations run for 10,000 years to assure the steady state conditions. We let the spin up simulations run for 10,000 years to assure the steady state conditions. We let the spin up simulations run for 10,000 years to assure the steady state conditions.
26. Roscher, C. et al. The role of biodiversity for element cycling and trophic interactions: an experimental approach in a grassland community. Basic Appl. Ecol. 10, 127–132 (2009).
27. Trumbore, S. Radiocarbon and soil carbon dynamics. Annu. Rev. Earth Planet. Sci. 37, 47–66 (2009).
28. Grace, J. B. Structural Equation Modeling and Natural Systems (Cambridge University Press, 2006).
29. Bessler, H. et al. Aboveground overyielding in grassland mixtures is associated with reduced biomass partitioning to belowground organs. Ecology 90, 1520–1530 (2009).
30. Lange, M. et al. Biotic and Abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. PLoS ONE 9, e96182 (2014).
31. Kramer, C. et al. Recent (< 4 year old) leaf litter is not a major source of microbial carbon in a temperate forest mineral soil. Soil Biol. Biochem. 42, 1028–1037 (2010).
32. Rasse, D. P., Rumpel, C. & Dignac, M. F. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant Soil 269, 341–356 (2004).
33. Baas, H. P., Weir, T. L., Ferry, L. G., Gilroy, S. & Vivancos, J. M. The role of root exudates in rhizosphere interactions with plants and other organisms. Annu. Rev. Plant Biol. 57, 233–266 (2006).
34. Bell, T., Newman, J. A., Silverman, B. W., Turner, S. L. & Lilley, A. K. The contribution of species richness and composition to bacterial services. Nature 436, 1157–1160 (2005).
35. Oelmann, Y. et al. Soil and plant nitrogen pools as related to plant diversity in an experimental grassland. Soil Biol. Biochem. 37, 720–729 (2005).
36. Spehn, E. M. et al. The role of legumes as a component of biodiversity in a cross-European study of grassland biomass nitrogen. Oekos 98, 205–218 (2002).
37. Zak, D. R., Holmes, W. E., White, D. C., Aaron, D. P. & Tilman, D. Plant diversity, soil microbial communities, and ecosystem function: are there any links? Ecology 84, 2042–2050 (2003).
38. Bessler, H. et al. Nitrogen uptake by grassland communities: contribution of N-2 fixation, facilitation, complementarity, and species dominance. Plant Soil 358, 301–322 (2012).
39. Ravenek, J. M. et al. Long-term study of root biomass in a biodiversity experiment reveals shifts in diversity effects over time. Oikos 123, 1528–1536 (2014).
40. Guenay, Y., Ebeling, A., Steinauer, K., Weisser, W. W. & Eisenhauer, N. Transgressive overyielding of soil microbial biomass in a grassland plant diversity gradient. Soil Biol. Biochem. 60, 122–124 (2013).
41. Kramer, C. & Gleixner, G. Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. Soil Biol. Biochem. 45, 425–433 (2013).
42. Liang, C. & Balsara, T. C. Microbial production of recalcitrant organic matter in global soils: implications for productivity and climate policy. Nat. Rev. Microbiol. 9, 75 (2011).
43. Schimel, J. P. & Schaeffer, S. M. Microbial control over carbon cycling in soil. Front. Microbiol. 3, 548 (2012).
44. Simpson, A. J., Simpson, M. J., Smith, E. & Kelleher, B. P. Microbially derived primers with enhanced specificity for badionyces—application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118 (1993).
45. White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62 (1979).
46. Stem, C. P. et al. A Language and Environment for Statistical Computing v. 3.0.3 (R Foundation for Statistical Computing, 2014).
47. Ros, S. et al. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol. 64, 795–799 (1998).
48. Lane, D. J. in Nucleic Acid Techniques in Bacterial Systematics. (eds Stackebrandt, E. & Goodfellow, M.) 115–175 (Willey, 1991).
49. Zhang, M. & Bruns, T. D. ITS primers with enhanced specificity for badionyces—a protocol for application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118 (1993).
50. White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62 (1979).
51. White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62 (1979).
52. Lane, D. J. in Nucleic Acid Techniques in Bacterial Systematics. (eds Stackebrandt, E. & Goodfellow, M.) 115–175 (Willey, 1991).
53. Zhang, M. & Bruns, T. D. ITS primers with enhanced specificity for badionyces—a protocol for application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118 (1993).
54. White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62 (1979).
55. White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia 40, 51–62 (1979).
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Additional information
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Author contributions
M.L., S.T. and G.G. designed the study; M.L. and G.G. wrote the manuscript; M.L. and N.E. performed statistical analyses; M.L. and C.A.S. modelled soil carbon; P.G.-M.-V., M.L., G.G. and J.R. performed the Ecotron experiment; A.A.M., B.T. and R.L.G. performed terminal restriction fragment length polymorphism analyses; N.E. and S.S. measured microbial activity; H.B. and C.E. measured root biomass. All authors reviewed and improved the manuscript.