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BRIEF REPORT

Diversity of organic amendments increases soil functions and plant growth

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Societal Impact Statement

Management practices, for example, in an agricultural context, are often tested in isolation or in pairwise interaction, but rarely using a higher number of jointly applied practices. In a proof-of-concept study, we test the effects of combining up to five management practices. Effects seen on soil and plant performance suggest that it may be worth to systematically and broadly examine the effects of higher order management combinations.

KEYWORDS
carbon diversity, factor interactions, management practices, plant growth, soil functions

1 | INTRODUCTION

With the growth of global population and climate change, both challenges to food security, there is an urgent need to prioritize the investigation of organic amendments options. Therefore, increasing sustainability of agriculture is of great importance, with the goal of producing high-quality food while minimizing resource consumption, ensuring environmental safety and crop yield (Reganold et al., 1990). Within the context of contributing to sustainable agriculture, numerous studies have investigated the impact of different types of carbon amendments in an attempt to increase soil carbon stock and soil fertility and potentially to boost crop yield. Prolonged use of conventional agricultural practices that heavily relies on synthetic fertilizers and intensive tillage can introduce soil acidification and nutrient imbalance, disrupt the soil microbial community and eventually lead to soil degradation. In contrast to many conventional agricultural practices, organic amendments introduce organic matter into the soil that can contribute to soil organic carbon (SOC), improving soil structure, water retention and nutrient availability. Importantly, studies have typically only focused on single amendment applications such as biochar, compost, wheat straw, cow dung or wool (Abdallah et al., 2019; Bonilla et al., 2012; Lu et al., 2020; Sun et al., 2017). Among the amendments, compost and biochar have been widely researched due to their positive impact on soil fertility, which refers to the soil's ability to support plant growth by providing essential nutrients, modifying soil structure and improving water retention and their local availability (Blackwell et al., 2012). Biochar is the product of pyrolysis of organic material, such as woody materials or crop residues, under oxygen limited environments. Biochar has large carbon sequestration potential in the long-term, increasing soil fertility and crop yield, and mitigating impacts from global change factors, such as drought and salinity (Akhtar et al., 2014; Dugdug et al., 2018; Lehmann & Joseph, 2015; Liang et al., 2014; Semida et al., 2019). Compost is a rather general term that refers to processed organic waste materials, such as wood chips, manure or a mixture. Straw amendments can increase nitrogen retention capacity in agricultural fields (Azam et al., 1991; Reichel et al., 2018). Cow dung and sheep wool amendments can increase phosphate solubilization, improve soil fertility and, consequently, increase crop yield (Gupta et al., 2016; Swain et al., 2012). Wool residue addition not only provides benefits in terms of waste recycling but also enhances aggregate stability, increases soil porosity and promotes plant growth (Abdallah et al., 2019; Ordiales et al., 2016; Palla et al., 2022).

While studying the effects of individual amendments is important for generating mechanistic understanding, it may also be beneficial to consider the joint application of multiple amendments. The reason is that the joint application of different amendments could lead to additive effects by combining individual effects and, potentially, by causing unexpected synergistic effects. Such mixed amendment effects have been studied for some pairs of the above-mentioned amendments. For example, combined application of biochar and compost can result in an increase of plant-available nutrients and improve soil water status in a short time (Liu et al., 2012). The joint application of biochar and other organic or inorganic fertilizers has been demonstrated to produce a synergistic effect, exceeding the performance of individual fertilizers alone, thereby stimulating crop yield (Bai et al., 2022). It has been hypothesized that carbon diversity may increase carbon persistence in the soil (Lehmann et al., 2020). However, mixed application of multiple amendments that include three or more amendments has not been studied yet.

With the goal of revealing joint effects of a large number of treatments, Rillig et al. (2019) developed an experimental design that used a random selection approach from a pool of factors, creating a gradient in the number of factors for which to examine effects. Based on the observation that the joint effect of two carbon amendments had beneficial effects on soil properties and plant growth, we conducted an experiment using an analogous 'random sampling' approach with the aim of examining effects of an increasing number of carbon amendments, with up to five carbon amendments used. However, increasing the number of amendments not only introduces a greater complexity but also multi-nutrient supplementation (with the risk of over-supplying nutrients beyond those recommended). We conducted the experiment primarily as a case study to explore effects of amendment interactions, not explicitly considering economic factors or regulatory frameworks. Our results suggest that farmers could supplement one amendment with multiple others, decreasing their reliance on any one carbon amendment.

Our hypothesis was that increasing carbon amendment diversity will introduce nutrient synergy and, subsequently, increase the activity of soil microbes and promote plant growth well beyond the effects of single amendments, and with the highest amendment number (in our case, five), delivering the greatest benefits.

2 | MATERIALS AND METHODS

2.1 | Soil preparation and seed germination

The tested soil was sandy loamy soil, collected in May 2023 from a conventional agricultural research field in the Brandenburg area (53.36°N, 13.80°E, Leibniz-Zentrum für Agrarlandschaftsforschung, Dedelow). Soil properties are reported in Notes S1. The soil was air-dried after collection from the site, homogenized and sieved (2 mm).

We chose red clover (*Trifolium pratense* L.), a common plant in seed mixes for grasslands in Germany, as the test plant. To prepare the rhizobium coating, rhizobium inoculants (Legume fix, Legume Technology) were mixed homogeneously with sterilized sand and placed in three sterilized containers. The seeds were disinfected and germinated in the sand-rhizobium inoculants mixture. Afterwards, all containers were placed in the climate chamber at 20°C for the seeds to germinate.

2.2 | Experimental design

Five carbon amendments were included as single factors ($N = 5$ each factor) in this experiment: biochar, compost, wheat straw, cow dung and sheep wool. The application dosage and product-related information are listed in Table 1. We further clarified the rationale of the selection of the five amendments in Notes S2.

All management practice treatments were added to plants with rhizobia. The control group ($N = 10$) we used to compare the effect sizes of different treatments was inoculated with rhizobia. To disentangle the mediation effect of rhizobia, we included a blank control ($N = 10$) for plants without rhizobium coating. However, this is beyond our research scope and is not discussed in this study.

Single amendments were tested with five replicates each, while treatments at the two factor level were randomly selected from the pool of five factors (without replacement for each experimental unit) with 10 replicates. For the five factor level, we tested eight replicates. The experiment thus consisted of 63 experimental units in total (Table S1). The factor combinations received the same level as the singled factors, that is, we did not adjust for the overall amount of carbon or material added. Among the 63 experimental units, one cow dung treatment, one sheep wool treatment and one five-factor treatment failed due to plant death during the incubation process.

TABLE 1 Tested organic amendments and concentrations.

Management practice	Product	Concentration (w/w)	Reference
Compost	Botanic garden Berlin- Dahlem	1.0%	(Liu et al., 2012)
Biochar	Carbovert, Eibenstock, Germany	0.5%	(Rajkovich et al., 2012); (Major, 2010)
Wheat straw	MultiFit, item no.: 1,008,159, Krefeld, Germany	0.2%	(Sun et al., 2017)
Cow dung	Bionaturplus, Germany	0.5%	(Tagele et al., 2023)
Wool	Bionaturplus, Germany	0.5%	(Palla et al., 2022)

The experiment was set up in 2 days. On the first day, 155 g air-dried soil was mixed manually with amendments in a sterilized box for 5 min and gently transferred to white cone-trainers (21 cm depth, 3.81 cm diameter at the top opening, 164 mL, SC10R, Ray Leach Cone-tainers, Stuewe and Sons Inc.). Afterwards, pots were watered from the top to achieve 60% water holding capacity. Two seedlings were transplanted on the second day to each pot and incubated in a climate chamber with a day/night temperature of 18/22°C, light/dark cycle of 6 a.m./9 p.m. and 50% relative humidity. After 1 week, pots were thinned to one seedling, and this day was counted as the first day of the experiment. To maintain 60% water holding capacity, we watered every 2 days during the first 2 weeks and on a daily basis from the third week to avoid water stress due to increased water demands of the test plants.

The experiment lasted 48 days. The harvest lasted 2 days, during which the above ground plant biomass was collected in 1 day, dried at 60°C and weighed afterwards. On the second day of harvest, belowground biomass was collected, washed and dried at 60°C; 5 g fresh soil was collected for enzyme activity measurement; and 20 g fresh soil was collected for soil respiration measurement in 50 mL mini bioreactors (Product Nr: 431720, Corning®, USA), which were stored for 3–4 days at 4°C before measurements. The remaining soil was air-dried for other measurements-water-stable aggregates and soil pH.

2.3 | Response variables

We tested 10 response variables to detect plant growth, soil functions and soil structure changes: above ground biomass, belowground biomass, rhizobium nodules counting, N-acetyl-glucosaminidase activity, cellulase activity, β-glucosidase activity, phosphatase activity, soil respiration, water-stable aggregates and pH.

To investigate plant growth, we measured above ground biomass, belowground biomass and rhizobium nodules. For the above ground biomass, we cut the plants at the bottom of the stem during harvest and dried them at 60°C. For the belowground biomass, we collected the visible main roots and collected the fine roots from the soil by hand (standardized time of 3 min). Roots were gently washed, and then, we counted the number of nodules on the fresh roots, considering only red mature nodules. Then, the roots were dried at 60°C. We weighed the dry mass using an analytical balance and recorded them as the above ground and belowground biomass.

We measured four enzyme activities and soil respiration to represent soil functions: β-glucosidase (carbon cycling), β-D-cellobiosidase (carbon cycling), β-N-acetylglucosaminidase (nitrogen cycling) and phosphatase (phosphorus cycling). To indicate soil structure, we measured water-stable aggregates for aggregates >250 μm and soil pH. The water-stable aggregates were measured with a wet-sieving machine (Eijkelkamp, Netherlands) following a well-established protocol (Kemper & Rosenau, 1986). Soil pH was measured with a pH metre (Hanna Instrument, Smithfield, USA) by mixing 5 g dry soil in 25 mL distilled water. We included the detailed measurements method in Notes S3.

2.4 | Statistical analysis

The statistical analysis in our experiment was conducted in R 4.2.1 (R Core Team, 2022). 'Tidyverse' (Wickham et al., 2019), 'dabestr' (Ho et al., 2019), 'Rmisc' (Hope, 2022), 'DImodels' (Moral et al., 2023) packages were used for data analysis and 'pheatmap' (Kolde, 2019) and 'ggplot2' (Wickham, 2016) were used to plot the figures.

The calculated effect sizes between treatments and control were bootstrapped with 100 permutations. We conducted significance tests with ANOVA and adjusted *p*-values with Tukey post hoc method. We listed all *p*-values for each response variable in Tables S2–S13.

We adapted the diversity interaction model using the 'DImodels' package, from which two models—factor identity model and pairwise interaction model—were included (Kirwan et al., 2009). In our experiment, M1 refers to the identity model, and it assumes amendments do not interact with each other. M2 is the pairwise interaction model, which considers both factor identity and all pairwise interactions.

We used the two models to predict each response variable at two-factor level and five-factor level and then bootstrapped 100 times to calculate the effect size. We compared the bootstrapped predicted effect size with the actual measured data to discover the model predictability.

3 | RESULTS

3.1 | Plant biomass

We measured total above ground and belowground biomass to capture plant growth responses to the treatments (Figure 1). For single factors, only straw slightly positively promoted plant and root growth. All other single factors had negative or neutral effect sizes when applied alone. However, when increasing the amendment level to five amendments, the effect size of above ground and belowground biomass were increased to 211.59 mg (95%-CI: 108.52 to 314.66) and 64.13 mg (95%-CI: 29.57 to 98.68). At the two-factor level, the factor identity model provided better predictability compared with the factor interaction model, while the factor interaction model performed better at the five-factor level.

3.2 | Soil functions and structure

In our experiment, we tested soil respiration, four types of soil enzyme activities to indicate soil functions (Figure 2a (1) to a (4)). β-glucosidase activity and cellulase activity did not show any significant impact, and the variation in-between treatments was limited. In contrast to control, phosphatase activity and N-acetyl-glucosaminidase activity had positive effect sizes for most of the treatments, where the effect sizes were significantly increased at the five factor level ($p < 0.001$ and $p = 0.0134$, respectively). At the single amendment level, wool had the highest effect size for phosphatase activity. From the perspective

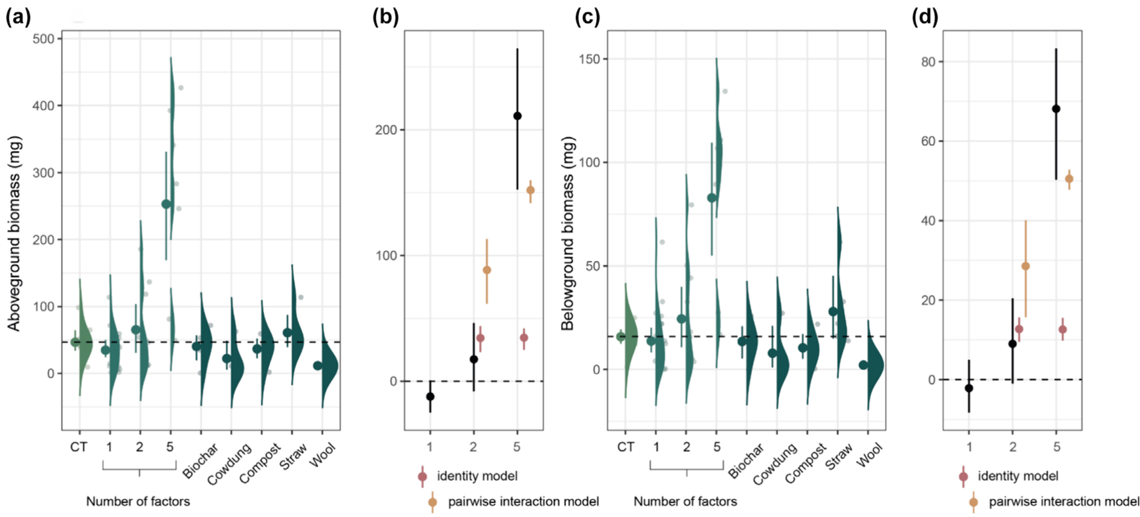

FIGURE 1 Above ground biomass and belowground biomass of single amendments and amendments at two- and five-factor level. Vertical lines refer to the 95% confidence intervals, and the dots in the middle indicate the mean values. In (a) and (c), the dots are individual replicates within each treatment, the dashed line is the mean of the control group. In (b) and (d), the lines indicate the bootstrapped effect size. The black colour is the raw data effect size, red colour refers to the effect size using the factor identity model to predict, and the orange colour refers to the effect size using the pairwise interaction model to predict.

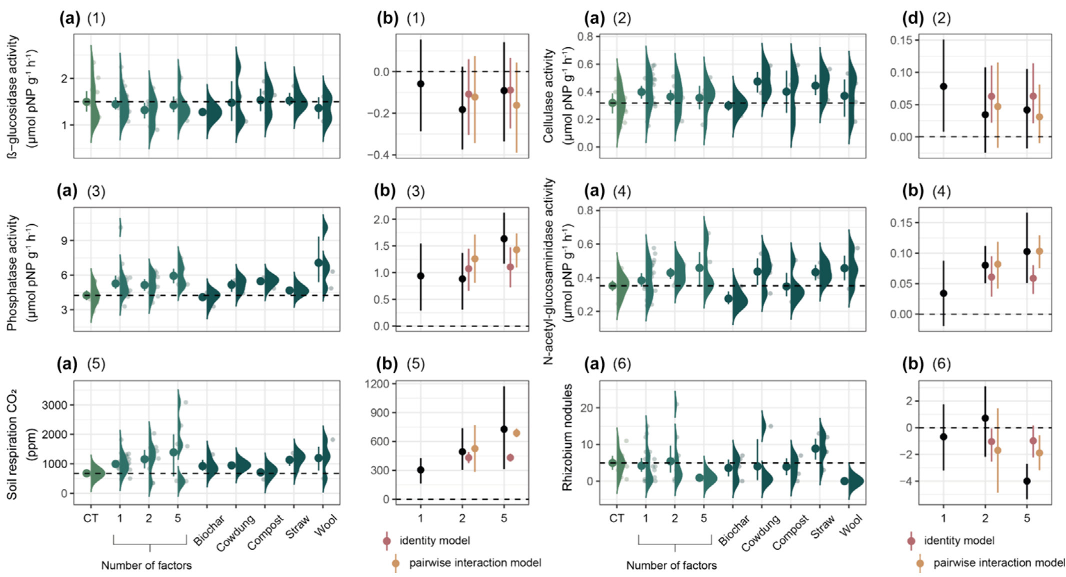

FIGURE 2 Soil functions response variables of single amendments and amendments at two- and five-factor level. Vertical lines refer to the 95% confidence intervals, and the dots in the middle indicate the mean values. In (a) (1) to (a) (6), the dots are individual replicates within each treatment, the dashed line is the mean of the control group. In (b) (1) to b (6), the lines indicate the bootstrapped effect size (permutation = 100). The black colour is the raw data effect size, red colour refers to the effect size using the factor identity model to predict, and the orange colour refers to the effect size using the pairwise interaction model to predict.

of model prediction, in contrast to the identity model, the interaction model provided closer predictions to actual data for cellulase activity and N-acetyl-glucosaminidase activity for both two and five amendment levels. On the other hand, the effect size of soil respiration at the two-factor level was close to the effect size of wool and straw, which were the highest effect sizes among the single amendments. Soil respiration at the five factor level tended to be highest but did not significantly differ from other treatments. For the rhizobium nodule count, treatments at five-factor level caused a negative effect size, in which no model can provide close predictability.

Water-stable soil aggregates, and pH were tested in this experiment (Figure 3). No significant results were found for any of the water-stable aggregate measurements, but the mean effect size tended to be lower for five amendments than in the other treatments. Among all treatments across various factor levels, wool showed the highest tendency towards enhancement of aggregate stability, while

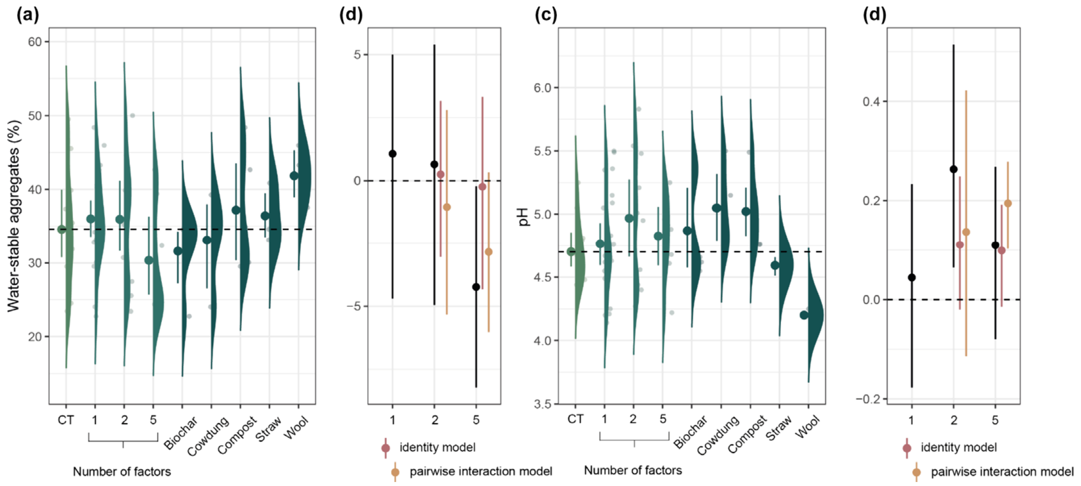

FIGURE 3 Soil structure response variables of single amendments and amendments at two- and five-factor level. Vertical lines refer to the 95% confidence intervals, and the dots in the middle indicate the mean values. In (a) and (c), the dots are individual replicates within each treatment, and the dashed line is the mean of the control group. In (b) and (d), the lines indicate the bootstrapped effect size (permutation = 100). The black colour is the raw data effect size, red colour refers to the effect size using the factor identity model to predict, and the orange colour refers to the effect size using the pairwise interaction model to predict.

biochar tended to decrease aggregate stability the most. In contrast with control, all treatments that contain multiple amendments led to a more alkaline soil pH. Among single amendment treatments, only straw and wool led to a more acidic soil. On the other hand, the identity model provided good predictability for aggregate stability for the two-factor level but not for the five-factor level. However, the model predictability is not in alignment with the pH—the identity model offered good predictability for the five-factor, but the pairwise interaction model gave a closer prediction for the two-factor level.

4 | DISCUSSION

We conducted a climate chamber experiment to test for amendment diversity effects. Our experiment revealed a substantial plant growth promotion in the five-amendment combination, while soil parameters were not responding in a similar manner.

4.1 | Carbon amendments effect

For above- and belowground biomass, all single factors had negative or slightly positive effects, while both above- and belowground biomass significantly increased at both the two- and five-factor levels. A disproportionate increase of plant biomass was detected at five-factor level, which clearly indicated that the amendment mixture had a strong beneficial effect on plant growth. To explore the mechanism driving this trend, we examined differences in both soil function and soil structure between treatments. All treatments modified soil pH with the exception of wool, while the increasing factor number did not have an effect. It thus appears that pH is likely not a factor explaining the plant growth response with a large number of factors applied. An exception is wool as a single amendment, a fibrous material primarily made of keratin, and it increased the phosphatase activity and N-cycling microbial activity but introduced a negative effect to plant biomass. A potential reason may be due to the nitrogen immobilization. Microbes use available nitrogen from the soil to balance the high carbon content in the wool decomposition process, causing nutrient competition between soil microbes and plants. The pH response cannot produce a direct explanation on the driving mechanisms of the plant biomass increase, but the overall modification of pH potentially contributed to the explanation of our hypothesis—carbon diversity promotes plant growth by boosting the microbial activities (Robson, 2012). A meta-analysis showed that organic amendment alone could accelerate the mineralization process of phosphorus primarily through optimizing soil physicochemical properties and stimulating microbial activity (Luo et al., 2019). The supplementation of organic amendments served as an energy source for soil microbes, such as for microbes harbouring phosphatase-encoding genes (e.g., phoD and phoC), and led to an increase of extracellular phosphatase activity. Moreover, the organic amendments modify soil pH towards neutrality, which is optimal for phosphatase activity. In our study, five amendments introduced both nutrient accumulation and amendments interaction. We considered the interactions in-between amendments that may have produced a synergistic effect, meaning, the diversity of the amendments may have contributed to the increase of phosphatase enzyme activity and N-cycling enzyme activity that was introduced by factor accumulation (Lehmann et al., 2011, 2020). However, in regards to the carbon-related enzyme-ß-glucosidase activity and cellulase activity, most of the effects were negative and not significant. This might be explained by sufficient available carbon in the soil, that is, microbes were not carbon-limited. Therefore, the increase of phosphatase and N-cycling enzymatic activity under sufficient available carbon, with the increase of soil pH towards neutrality, may have promoted phosphorus and nitrogen mineralization, which resulted in an increase of plant-available phosphorus and nitrogen and thus substantially promoted plant growth (Coleman et al., 2018).

4.2 | Diversity interaction model performance

In both above- and belowground biomass, the prediction of M1 indicated that the carbon amendment identity effect was strong at the two-factor level. In comparison to the actual data, the higher prediction values of M2 at the two-factor level implied that the pairwise joint effect overestimated the effects. In contrast, the factor identity model provided a closer prediction to the actual data. However, at the five-factor level, the identity model M1 cannot provide any prediction, and the interactive model M2 brings the prediction closer to the actual data. M2 considered both factor identity and factor interactions, but the mean value of the prediction at five-factor level is still below the mean value of the actual data; this implied that a more complex synergistic effect on plant biomass was introduced when all five amendments were added.

In regards to soil functions, we noted that M2 provided better predictions than M1 for both two-factor and five-factor levels in soil respiration. This implies that the soil microbial community may strongly be influenced by the pairwise factor interactions, which may contribute to the explanation of the synergistic effects of plant biomass under multiple amendments (Kirwan et al., 2009).

5 | CONCLUSIONS

We conclude that more diverse carbon amendments may benefit plant growth and potentially, in the long-term, improve soil health. Based on the observations from this exploratory study, we suggest a shift in carbon amendment research towards also exploring effects of more complex soil amendments, such as the combination of multiple amendments, in addition to the single-treatment or pairwise interaction effects (Rillig et al., 2024). As a next step, it will be crucial to investigate the potential mechanisms driving such complex interactions and to understand under what conditions such effects can occur. Additionally, the potential of such a mixture to mitigate the negative effects of multiple global change factors on different crops should be tested further (Peláez-Vico et al., 2024; Sinha et al., 2024).

AUTHOR CONTRIBUTIONS

Huiying Li: Design of the study; experiment setup; analysis of data; and writing. **Anika Lehmann and Rebecca Rongstock**: Review and editing. **Yaqi Xu and Edda Kunze**: Experiment setup. **Peter Meidl**: Editing. **Matthias C. Rillig**: Conceptualization; review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in figshare under 10.6084/m9.figshare.25933948.v1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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