



Combined addition of chemical and organic amendments enhances plant resistance to aboveground herbivores through increasing microbial abundance and diversity

Linhui Jiang^{1,2} · Michael Bonkowski³ · Ling Luo^{1,2} · Paul Kardol⁴ · Yu Zhang^{1,2} · Xiaoyun Chen^{1,2} · Daming Li⁵ · Zhenggao Xiao^{1,2} · Feng Hu^{1,2} · Manqiang Liu^{1,2}

Received: 24 February 2020 / Revised: 16 April 2020 / Accepted: 28 April 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

Two greenhouse experiments using soils from long-term field plots were carried out to test whether and how soil factors modulated by organic amendments feed back to rice plant growth and defense against an aboveground herbivore, the planthopper *Nilaparvata lugens*. Using factorial combinations of sterilized soil and soil inocula obtained from chemically amended plots (i.e., control treatment) or chemically plus organically amended plots (i.e., organic treatment), we disentangled the effects of biotic and abiotic soil properties on plant and planthopper performance. We found that, compared with abiotic soil properties, soil biological factors were the main drivers in regulating plant growth performance. Specifically, soil biota that are shaped by the organic treatment had high microbial abundance and diversity and enhanced rice plant tolerance (i.e., increasing plant total biomass) and resistance (i.e., decreasing amino acid and sugar concentrations) to planthoppers. Moreover, the organic treatment simultaneously increased plant growth and defense against planthoppers, which could be explained by high soil nutrient availability driven by soil biota. Our results demonstrate the importance of synergistic effects of soil biota and soil abiotic factors on plant growth and resistance to herbivory. These findings are important for better understanding the mechanisms and impacts of ecological intensification as well as the potential of steering soil communities to reduce the use of chemical fertilizers and pesticides and further optimize crop production.

Keywords Organic amendment · Soil biodiversity · Sustainable agriculture · Above- and belowground · Herbivore · Plant defense

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00374-020-01473-w>) contains supplementary material, which is available to authorized users.

✉ Manqiang Liu
liumq@njau.edu.cn; manqiang-liu@163.com

- ¹ Soil Ecology Lab, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China
- ² Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization & Jiangsu Key Laboratory for Solid Organic Waste Utilization, Nanjing 210095, China
- ³ Cluster of Excellence on Plant Sciences (CEPLAS), Terrestrial Ecology, Institute of Zoology, University of Cologne, 50674 Köln, Germany
- ⁴ Department of Forest Ecology and Management, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden
- ⁵ Jiangxi Institute of Red Soil, Nanchang 331717, China

Introduction

The functioning of soil ecosystems is facing serious challenges under the increasing pressure of anthropogenic activities (Grime et al. 2000; Sala et al. 2000). Soil communities are integral in ecosystem responses to perturbations and management actions (Bardgett and van der Putten 2014; Delgado-Baquerizo et al. 2017; Singh and Gupta 2018), but their key roles in sustaining the ecological processes in agricultural management strategies remain little understood. Evidence is mounting that soil communities can not only enhance plant tolerance to herbivores but also induce systemic resistance against herbivores and pathogens (Martinez-Medina et al. 2016; Pineda et al. 2010; Raaijmakers and Mazzola 2015). Thus, targeted management of soil communities might be an effective approach to enhance agricultural sustainability (Xu et al. 2020).

In agroecosystems, soil community composition and function depend on the input of plant-derived organic compounds

but is also influenced by chemical amendments (e.g., industrial inorganic fertilizer) and organic amendments, such as manure and biochar (Ikoyi et al. 2020; Liu et al. 2020; Wang et al. 2019a). Comparisons between organic and chemical amendments have received much recent attention, in particular the negative impacts of chemical amendments on soil degradation, environmental pollution, and aboveground pest outbreaks (Gravuer et al. 2019; Knapp and van der Heijden 2018; Shi et al. 2020). Previous studies have shown that soil chemical properties affected by fertilization regimes could influence crop nutrient uptake and growth rate. Moreover, soil biological factors, including microbial abundance and diversity, also play vital roles in maintaining soil functions via C and nutrient turnover (Bedada et al. 2014; Singh and Gupta 2018). However, how biotic and abiotic soil factors are driven by different fertilization regimes and in turn affect plant growth and resistance to aboveground pests is largely unknown.

With the development of more sustainable agricultural practices, increasing attention has been paid to the influence of soil communities on plant performance and defense against herbivores (Friesen et al. 2011; Heinen et al. 2018, 2020; Lau and Lennon 2012; Pineda et al. 2020; Qiao et al. 2019). For example, targeted manipulation of soil biota can stimulate soil life and maximize the biological processes they drive, and hence, their contribution to sustainable ecosystem functioning, and thereby increasing crop yield (Bender et al. 2016; Ma et al. 2020; Mariotte et al. 2018). A diverse soil community can also promote the uptake of micronutrients by plants and is essential in keeping a plant's nutrient balance (Chaboussou 2004). Moreover, soil biota can positively affect plant defense against herbivores via stimulating the synthesis of plant secondary compounds (Pineda et al. 2013; Rashid and Chung 2017), and improving phytohormone signaling, such as salicylic (SA) and jasmonic acid (JA) signaling, through the regulation of defense genes (Puga-Freitas and Blouin 2015; Spence et al. 2014; Zhu et al. 2018). In this context, managing plant-associated microbes might provide a sustainable solution to decrease agricultural pollution by increasing crop yields, and reducing the use of pesticides. However, so far, most studies on aboveground plant defense have focused on the effects of individual microbial species or strains. There is an urgent need to further explore what factors and processes induced by complex soil communities are involved in these interactions (Pineda et al. 2017).

Plants can employ various strategies to maximize their fitness against herbivore attack, including tolerance and resistance. Generally, tolerance is the ability of plants to regrow and reproduce in the presence of herbivores (Strauss and Agrawal 1999), whereas resistance is the ability of plants to reduce herbivore preference or performance (Karban and Baldwin 1997). Previous studies have shown that increased investment in defense may come at the expense of plant

growth (i.e., the growth-defense hypothesis, Fig. 1a; Bechtold et al. 2018; Huot et al. 2014), leading to differential investment in plant growth or defense depending on, for example, soil conditions. Here, the resource availability hypothesis postulates that resource availability is the major determinant of the plant growth-defense trade-off (Fig. 1b; Coley et al. 1985; Züst and Agrawal 2017). Therefore, plants might exhibit faster growth rates and lower defense levels in environments with high resource availability.

In agroecosystems, to enhance crop production, fertilizers are applied to improve soil nutrient availability. Although the application of chemical fertilizers can promote crop yield, it causes major problems (Foley et al. 2011; Reganold and Wachter 2016). Besides severe environmental problems, excessive use of chemical fertilizers can lead to outbreak of pest herbivores, due to nutrient imbalances and lower pest resistance (the mineral balance hypothesis; Alyokhin et al. 2005; Phelan et al. 1996). The mineral balance hypothesis (Fig. 1c) suggests that soil organic matter (SOM) and microbial activity associated with organic amendments help to maintain the nutrient balance in crops, for example through promoting protein synthesis and inhibiting the accumulation of plant amino acids. Hence, adding organic amendments in addition to chemical fertilizers is one of the recommended ways to both solve the problems derived from excessive chemical fertilizers and sustain crop yield (Ji et al. 2020; Liu et al. 2009; Muller et al. 2017). Nevertheless, the role of abiotic and biotic soil properties as affected by organic amendments in plant growth-defense trade-off remains poorly understood.

Here, we utilized a long-term fertilization experiment to set up two greenhouse experiments examining whether and how biotic and abiotic soil properties as influenced by organic amendments alter rice plant growth and defense against aboveground herbivores. First, we investigated the soil legacies of organic amendments on rice plant and herbivore performance using a fully factorial experiment. We hypothesized that (H1) plants with higher biomass and lower nutrients in organically amended soils would possess greater tolerance and resistance to herbivores, and thereby suffer less herbivore abundance than plants grown in control soils. Second, a reciprocal transplant experiment was conducted to disentangle the effects of biotic and abiotic soil properties as influenced by organic amendments on plant-herbivore interactions. We hypothesized that (H2) compared with the sterilized soil treatments, the presence of soil biota, especially when shaped by organic amendments, could promote plant growth and enhance plant tolerance against herbivores. We expected that soil biota are the main drivers in regulating plant growth performance, rather than abiotic soil properties. We further hypothesized that (H3) high soil nutrient availability driven by soil biota in soils receiving organic amendments would promote plant resource investment in growth rather than in defense against herbivores.

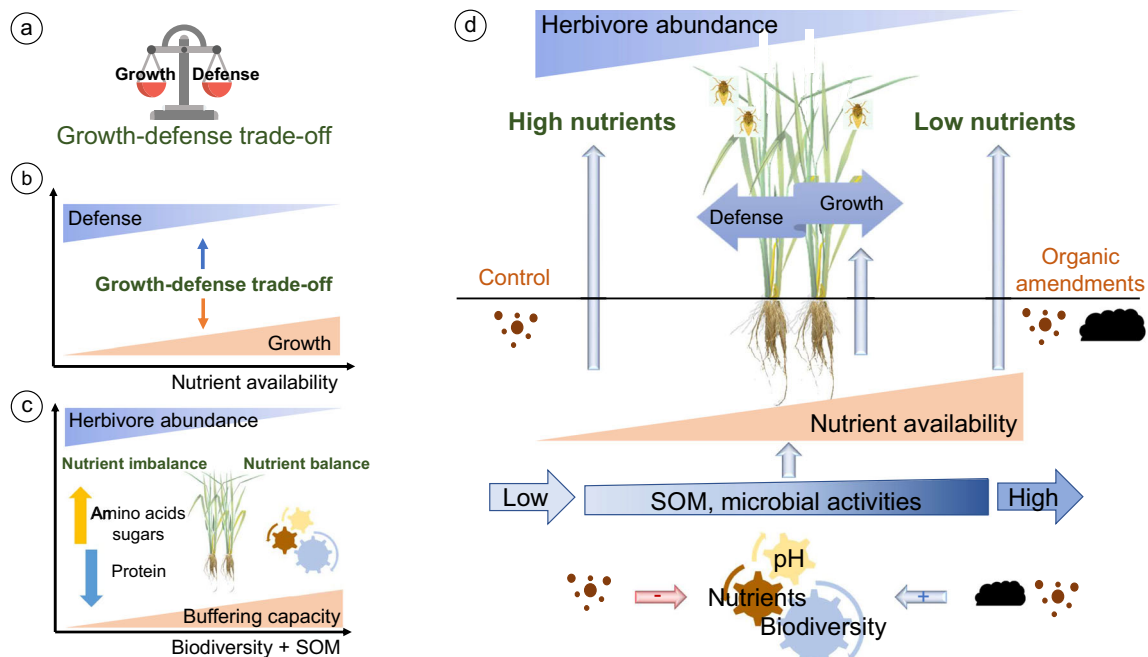


Fig. 1 Conceptual diagram illustrating (a) the growth-defense hypothesis. This hypothesis predicts that activation of defense mechanisms comes at the expense of plant growth as plants typically are resource-limited; (b) the resource availability hypothesis. This hypothesis posits that resource availability is the main driver of the plant growth-defense trade-off. Plants tend to grow faster but possess lower levels of defense compounds in environments with high resource availability; (c) the mineral balance

hypothesis. This hypothesis suggests that SOM and microbial activities associated with organic amendments promote a buffering capacity that can maintain the nutrient balance in crops, thus increasing plant resistance to herbivores. **d** Conceptual diagram of how biotic and abiotic soil properties as affected by organic treatment influence rice growth and susceptibility to herbivores and herbivore performance

Materials and methods

Soil collection and characterization

Soil samples were collected from a rice paddy field experiment located at the Jiangxi Institute of Red Soils (28° 15' N, 116° 20' E, Jiangxi Province, China) to set up two greenhouse experiments. Mean annual temperature in the study area is approximately 17.2 °C. Mean annual precipitation and evaporation are approximately 1550 and 1150 mm, respectively. The soil is classified as a typical Stagnic Anthrosol (IUSS Working Group 2006), silty loam texture (19.7% sand, 48.5% silt, and 31.8% clay). Before establishment of the experiment, the field had been used for rice (*Oryza sativa* L.) cultivation for at least 100 years.

The long-term fertilization experiment was established in 1981 with a double rice cropping system (rice-rice-winter fallow). The experiment consists of a randomized design with three replicates of nine treatments (all plots were 46.67 m²), aiming to determine the optimum ratio of chemical fertilizers and the role of organic amendments. The details of the field experiment have been described in Huang et al. (2009). In 2014, to estimate the effects of organic amendments on plant-herbivore interactions, we selected two treatments with distinct fertilization regimes, i.e., only chemical amendments (hereafter, control

treatment, C), and combined addition of chemical and organic amendments (hereafter, organic treatment, O). The C treatment consisted of addition of NPK fertilizer (N: 90 kg ha⁻¹, P₂O₅: 45 kg ha⁻¹, K₂O: 75 kg ha⁻¹) for each rice cycle, while the O treatment consisted of the same amount of NPK fertilizer plus green manure (22,500 kg ha⁻¹; organic C: 467 g kg⁻¹, total N: 4.0 g kg⁻¹, total P: 1.1 g kg⁻¹, total K: 3.5 g kg⁻¹) for the early rice cycle and NPK fertilizer plus pig manure compost (22,500 kg ha⁻¹; organic C: 340 g kg⁻¹, total N: 6.0 g kg⁻¹, total P: 4.5 g kg⁻¹, total K: 5.0 g kg⁻¹) for the late rice cycle. We then used the C and O soils to set up two greenhouse experiments exploring the contributions of soil biota to plant growth performance as influenced by the addition of organic amendments.

In May 2014, three soil cores per plot were sampled using a standard core sampler (diameter: 5 cm) to a depth of 20 cm. We pooled the samples from the C treatments and we pooled the samples of the O treatments into two composite samples, i.e., C and O soils. The method of pooling soil samples has some drawbacks (Reinhart and Rinella 2016), but the purpose of our study was to test the average effects of biotic and abiotic soil properties as influenced by organic amendments on plant-herbivore interactions, and we were not interested in the spatial variability of soil community effects (Cahill et al. 2017; Gundale et al. 2017; Teste et al. 2019). The composite soil

samples were then passed through a 5-mm sieve. Each soil sample was divided into three parts: (1) half of each sample was sterilized by gamma-irradiation (50 kGy) and used as a substrate for rice growth in the second experiment. (2) The largest part of the remaining soil sample was not sterilized and was used to investigate the legacy effects of organic treatment on rice plant growth in the first experiment. (3) A small part of each sample was used as an inoculum in the second experiment by shaking the soils at a 2:1 water-to-soil ratio (Rodríguez-Echeverría et al. 2013). We used the soil suspensions as inocula without filtering, since filtering would exclude microorganisms attached to soil particles or soil organisms too large to pass through the mesh (van de Voorde et al. 2012). In addition, 10 g soil subsamples were used for analyses of soil microbial communities (see below), and 200 g soil subsamples were used to measure the pH and nutrient content (see below). Soil samples were stored at -80°C and 4°C for microbial and chemical properties, respectively. Chemical properties of non-sterilized and sterilized soils are shown in Table 1.

Rice cultivar and sap-feeding herbivores

In our study, we used the local rice cultivar Nongxiang 98. Seeds were surface-sterilized in 10% H_2O_2 (w/w) solution for 30 min, rinsed five times, and then soaked in sterile distilled water for 12 h before being germinated on damp gauze at 28°C . As sap-feeding herbivore, we selected the specialist planthopper *Nilaparvata lugens* (Stål) (Hemiptera, Delphacidae), which is one of the most economically deleterious pests in rice cultivation (Bottrell and Schoenly 2012). It sucks the phloem sap of rice plants and causes plants to wilt and turn brown (Yadav and Chander 2010). In a previous study, we have shown that our rice cultivar was medium susceptible to this herbivore (Huang et al. 2012). Planthoppers were collected from paddy fields and then reared on young seedlings of Nongxiang 98 under a 16:8 h light:dark photoperiod at 15,000 lx, at $26 \pm 1^{\circ}\text{C}$, and at a relative humidity of $66 \pm 5\%$.

Experimental set-up

Experiment I: effects of organic amendments on plant growth and resistance to aboveground herbivores

The first experiment was a fully factorial experiment, in which soil treatments (C or O) and *N. lugens* (presence or absence) were factors, resulting in four treatments with five replicated pots per treatment. During the experiment, midday temperatures in the greenhouse ranged from 25 to 30°C , and natural light was provided. A total of 20 polypropylene pots (250 mL) were filled with 200 g (dry weight equivalent) of C or O soils, and each pot was planted with six 1-week-old rice seedlings. All pots were located randomly in the greenhouse, and plants were not affected by any other herbivores. Seven weeks after transplanting, fourth instar nymphs of the planthopper *Nilaparvata lugens* were added to half of the pots (i.e., five pots per soil treatment, ten pots in total). We established a low to moderate infestation density based on an earlier study (Huang et al. 2013) with the expectation of severe herbivore attack but no plant mortality. Each pot received a total of 30 nymphs. The planthoppers were contained in the pots by transparent polyvinyl chloride (PVC) cylinders, higher than the plants ($d = 20$ cm, $h = 60$ cm), covered with a gauze. Pots without herbivores were also equipped with PVC-cylinders, to ensure similar conditions to all plants. One day after transfer, planthoppers were counted and any losses were replaced. All pots were watered daily until 3 days before the final sampling. The experimental pots were harvested 3 weeks after *N. lugens* infestation, when rice plants showed significant herbivore damage.

Experiment II: effects of biotic and abiotic soil properties on plant growth and resistance to aboveground herbivores

Experiment II consisted of two phases, a conditioning phase (to stabilize microbial communities introduced by the inoculum) and a test phase (Fig. 2). In the conditioning phase, 60 pots were set up by weighing 350 g (dry weight equivalent) of sterilized soils into 500-mL glass pots under laminar air-flow conditions; 30 pots contained sterilized O soils and 30 pots contained sterilized C

Table 1 Chemical properties of non-sterilized and sterilized field soils. Values shown are means \pm SE ($n = 5$)

	Non-sterilized soils		Sterilized soils	
	Control	Organic	Control	Organic
Exchangeable $\text{NH}_4^+\text{-N}$ (mg kg^{-1})	2.18 ± 0.17	4.34 ± 0.23	37.23 ± 0.33	47.21 ± 0.43
$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	4.72 ± 0.21	3.16 ± 0.11	4.69 ± 0.36	3.40 ± 0.03
DOC (mg kg^{-1})	103.34 ± 4.86	158.38 ± 4.61	369.74 ± 12.75	487.49 ± 19.45
AP (mg kg^{-1})	8.73 ± 0.44	46.20 ± 2.64	21.88 ± 0.24	115.34 ± 2.25
AK (mg kg^{-1})	130.27 ± 4.83	174.30 ± 2.32	258.42 ± 15.36	442.06 ± 10.11
pH (H_2O)	6.05 ± 0.14	6.26 ± 0.07	5.70 ± 0.09	5.95 ± 0.12

DOC dissolved organic C, AP available P, AK available K

soils. For each of the sterilized soils, 10 pots were inoculated with organic soils (15 mL pot⁻¹, approximately 2% of dry weight; O + o and C + o), 10 pots with control soils (15 mL pot⁻¹, approximately 2% of dry weight; O + c and C + c), and 10 pots with sterile distilled water (15 mL pot⁻¹, O + s and C + s). This resulted in a total of six soil treatments (O + o, C + o, O + c, C + c, O + s, and C + s) with ten replicated pots per soil treatment (Fig. 2). All soils were then adjusted to and maintained at 80% water-holding capacity using sterilized distilled water. The pots were sealed with rubber plugs (to avoid contamination from airborne microorganisms) and incubated in darkness at 25 °C for 8 months for the soil communities to establish and stabilize (Griffiths et al. 2001). During the incubation period, all pots were vented in a laminar air-flow cabinet for 1 h to reset them to incubation conditions when CO₂ concentrations in the pots were beyond 5% (Ma et al. 2015). At the end of the incubation period, a sample of 80 g was collected from each pot for chemical and soil microbial analyses (see below). The remaining soil of each pot was used in the test phase of this experiment.

The test phase of the experiment was conducted in the same greenhouse as the conditioning phase, but under natural light conditions. A total of 60 polypropylene pots (250 mL) were filled with 200 g of soil (dry weight equivalent) collected from the conditioning phase (the 6 soil treatments: O + o, O + c, C + o,

C + c, O + s, and C + s; 10 pots per treatment), and each pot was planted with 6 1-week-old rice seedlings under laminar air-flow conditions (Fig. 2). Each pot was sealed in a 30 cm × 40 cm polyethylene sterile bag to avoid contamination from airborne microorganisms. The bags were opened in a laminar air-flow cabinet to flush with ambient air for 1 h three times a week. Three weeks after transplanting, the polyethylene sterile bags were removed to ensure sufficient space for plant growth. Seven weeks after plant transplanting, fourth instar nymphs of the planthopper *Nilaparvata lugens* were added to half of the pots (i.e., 5 pots per soil treatment, 30 pots in total). Each pot received a total of 30 nymphs. All the pots were also equipped with PVC-cylinders, to ensure similar conditions to all plants. One day after transfer, planthoppers were counted and any losses were replaced. All pots were watered daily until 3 days before the final sampling. The experimental pots were harvested 3 weeks after *N. lugens* infestation.

Measurements

Soil chemical properties

We measured chemical properties of both sterilized and non-sterilized field soils and soil samples at the end of conditioning

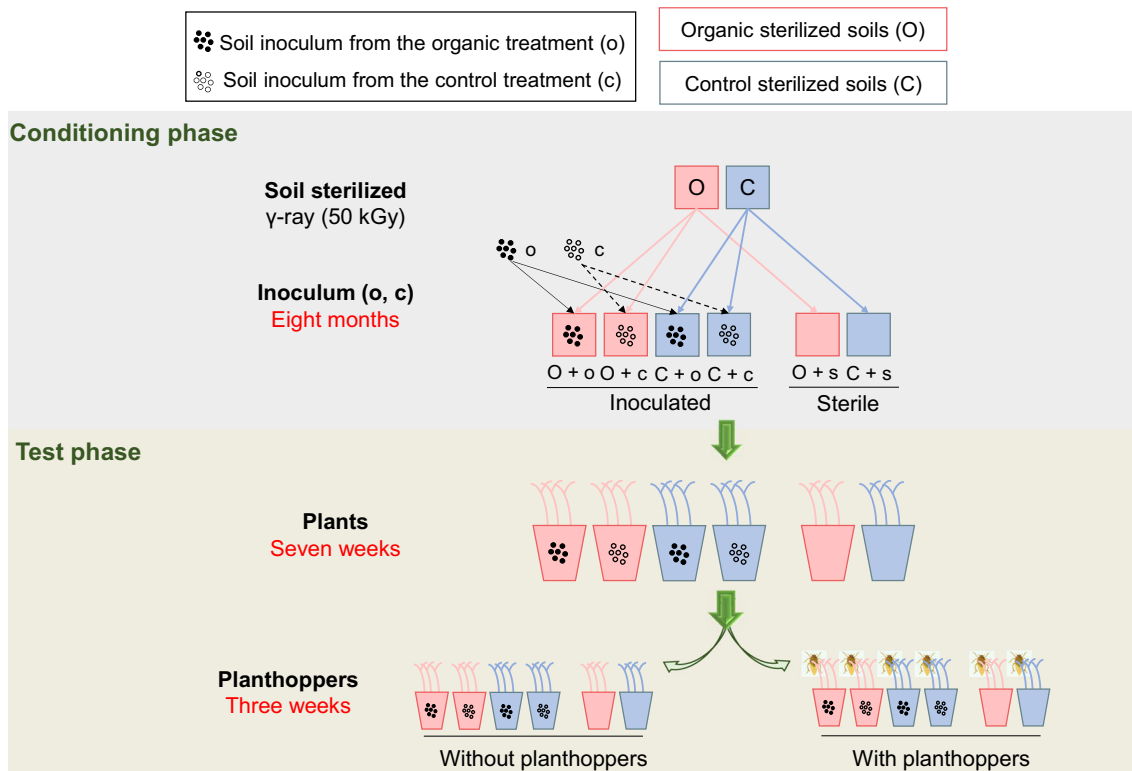


Fig. 2 Schematic illustrating the design of experiment II. In the conditioning phase, long-term organically amended soils (O) and control soils (C) were sterilized and then inoculated with soil inoculum from the organic treatment (o) or the control treatment (c). The design also included sterilized soils without inoculum, resulting in six different soils with

ten replicated pots per treatment. In the test phase, six 1-week-old rice seedlings were transplanted to each pot. Seven weeks later, nymphs of the planthopper *Nilaparvata lugens* were added to half of the pots (30 nymphs per pot); the herbivore treatments consisted of five replicated pots

phase of experiment II. Mineral N (exchangeable NH_4^+ -N and NO_3^- -N), dissolved organic N (DON), and dissolved organic C (DOC) were extracted from 10 g fresh soil with 50 mL ultrapure water (shaken for 1 h at 20 °C at a speed of 200 rev min^{-1}) and then centrifuged at 3800 g for 10 min. The solution was filtered through a 0.45 μm cellulose acetate membrane and the flow-through was analyzed with a total C analyzer (Elementar, Langensfeld, DE) for the DOC concentration and a continuous flow analyzer (Skalar San++, Breda, the Netherlands) for exchangeable NH_4^+ -N, NO_3^- -N, and DON concentrations. Soil available P (AP) was extracted with 0.50 M NaHCO_3 and determined colorimetrically using molybdate. After extraction with neutral ammonium acetate, soil available K (AK) concentrations were determined using a flame photometric method. Soil pH was measured with a PHS-3C mv/pH detector (Shanghai, China) using a 1:5 soil-to-water ratio.

Soil microbial communities

After the conditioning phase in experiment II, DNA was extracted from 0.5 g of soil (fresh weight) from each pot using the Fast DNA® SPIN kit for soil (MP-BIO, Santa Ana, California, USA) according to the manufacturer's instructions. Quality and concentration of extracted DNA were evaluated by 1% agarose gel electrophoresis and measured with a NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA). Following a robust, cost effective and widely used analysis method for microbial community composition (Almasia et al. 2016; Gong et al. 2019; Trivedi et al. 2019), we evaluated the genetic composition and richness of bacterial and fungal communities in different soils by terminal restriction fragment length polymorphisms (T-RFLP) and quantified the abundance of bacterial and fungal taxa by quantitative real-time PCR (qPCR). A detailed description of the molecular analyses is provided in Method S1 in the Supporting Information.

Herbivore and plant performance

In both experiments, herbivore and plant performance were measured 3 weeks after *N. lugens* infestation. *N. lugens* nymphs were collected from the plants according to Huang et al. (2012), counted, and their biomass per pot was determined after drying at 60 °C for 48 h. The average shoot height (from the stem base to the longest leaf top) of the six plants was calculated for each pot. Afterwards, plant shoots and roots were harvested. Fresh shoot and root biomass were recorded, and half of each sample was dried at 65 °C for 72 h to obtain dry weight. The remaining fresh sample was used for plant nutritional metabolite (soluble sugars and amino acids), secondary metabolite (phenolics), and phytohormone (salicylic and jasmonic acid, hereafter SA and JA) measurements.

The shoot soluble sugars were determined by the sulfuric acid-phenol colorimetric method according to Masuko et al. (2005) and D'Costa et al. (2014), total amino acids were measured according to Rohsius et al. (2006) with some slight modifications, and phenolics were determined using the Folin-Ciocalteu colorimetric method (Ainsworth and Gillespie 2007) as described previously (Xiao et al. 2016, 2019). The accumulation of the shoot phytohormones SA and JA was measured using a modified HPLC-MS/MS method (Hettenhausen et al. 2013; Pan et al. 2010). A detailed description of the phytohormone measurements is provided in Method S2 in the Supporting Information.

Data analysis

In both experiments, as a proxy for plant tolerance to herbivory, tolerance scores were calculated as the ratio of the shoot biomass of damaged plants relative to the mean value of undamaged plants under the same treatment conditions (Huang et al. 2010; Simms and Triplett 1994). Plant resistance was estimated by counting the numbers of *N. lugens* (ind. pot^{-1}); here, lower numbers of *N. lugens* mean that plants possess higher resistance to herbivory. Besides, linear correlation analyses were used to evaluate the relationships between plant growth (as estimated by rice plant total biomass) and resistance (as estimated by numbers of *N. lugens*).

In experiment II, we used two-way ANOVA to test the effects of biotic and abiotic soil properties on numbers and biomass of *N. lugens*. Soil inoculum and sterilized soil treatments were included as fixed factors. These analyses were performed using IBM SPSS Statistics 22. We then used principal coordinate analysis (PCoA) based on weighted UniFrac distance metrics (Lozupone et al. 2006) to explore the dissimilarities of the microbial (bacterial or fungal) community composition using R Version 3.6.0. To identify which plant traits (total biomass and the concentrations of amino acids, sugars, phenolics, SA, and JA) were the best predictors of *N. lugens* abundance, we conducted a classification random forest analysis (Breiman 2001) as explained in Delgado-Baquerizo et al. (2015). Random forest adds an additional layer of randomness to bagging, and each tree used a different bootstrap sample of data (5000 trees). These analyses were conducted using the "randomForest" package in R (Liaw and Wiener 2002). The significance of the model and the cross-validated R^2 values were assessed with 5000 permutations of the response variable (i.e., *N. lugens* abundance) using the "A3" R package (Fortmann-Roe 2013). Similarly, the significance of each predictor (here, plant traits) on *N. lugens* abundance was assessed by using the "rfPermute" package (Archer 2016). Additionally, another classification random forest analysis was employed to identify the best predictors of plant total biomass. In the second random forest analysis, the different abiotic (soil pH, exchangeable NH_4^+ -N, NO_3^- -N, DON,

DOC, AP, and AK) and biotic (i.e., bacterial and fungal abundance and diversity) soil properties were included as predictors of plant total biomass.

To test for the effects of soil inoculum (s, o, or c) and sterilized soil treatment (O vs. C), and their interaction on plant growth (shoot biomass and root biomass) and defenses (amino acids, sugars, phenolics, SA, and JA) in the presence of planthoppers, we performed two-way permutational multivariate analyses of variance (PERMANOVA) using the *adonis* function in the “vegan” package in R (Oksanen et al. 2018). PERMANOVAs were run based on Bray-Curtis dissimilarities with 999 permutations. We then estimated partial eta-squared effect sizes—the relative importance of the two manipulated factors (sterilized soil treatments and soil inocula)—on rice growth and defenses in the presence of planthoppers (Glassman et al. 2018; Ialongo 2016). In addition, linear correlation analyses were used to evaluate the relationships between *N. lugens* and plant total biomass, the relationships between *N. lugens* and plant nutrients (concentrations of shoot amino acids and sugars), the relationships between plant total biomass and microbial properties (bacterial and fungal abundance and diversity), and the relationships between plant nutrients and microbial properties. To visualize the associations among abiotic (pH, exchangeable $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, DON, DOC, AP, and AK) and biotic (bacterial and fungal abundance and diversity) soil properties, plant traits (total biomass, root-to-shoot ratio, and the concentrations of amino acids, sugars, phenolics, SA, and JA), and herbivore performance (*N. lugens* number), we conducted a principal component analysis (PCA) using R to show the effects of different soil treatments on abiotic and biotic soil properties, plant traits, and planthopper numbers.

Results

Experiment I

Compared with the control, the organic treatment significantly increased plant total (shoot and root) biomass, by ~72% and ~83% in the absence and presence of planthoppers, respectively ($p < 0.05$, Fig. 3a, b, Table S1). Plants grown in organic soils had ~82% higher and ~21% lower concentrations of amino acids than plants grown in control soils in the absence and presence of planthoppers, respectively (Fig. 3c). In the presence of planthoppers, the organic treatment significantly reduced shoot SA and JA concentrations by up to ~60% and ~38%, respectively, compared with the control ($p < 0.05$, Fig. 3g, h). In addition, the organic treatment significantly decreased planthopper numbers by ~42% as compared to the control ($p < 0.05$, Fig. 3i). There was no significant correlation between planthopper numbers and plant total biomass in either control or organic treatments (Fig. 3j).

Experiment II

Biotic and abiotic soil properties after the conditioning phase

Both soil treatment and soil inoculum significantly affected the composition of bacterial and fungal communities (Fig. 4a, b). Compared with the control, the abundance and diversity of bacteria and fungi was increased in organic soils ($p < 0.05$, Fig. 4c–f, Table S2). Soil inoculum from the organic treatment caused higher microbial abundance (~2.3% for bacteria and ~0.7% for fungi) and diversity (~23% for bacteria and ~18% for fungi) than soil inoculum from the control treatment when added to sterilized organic soils (Fig. 4c–f), and higher fungal abundance (~1.9%) and diversity (~10%) when added to sterilized control soils (Fig. 4d, f). Similarly, the organic treatment also increased the content of exchangeable $\text{NH}_4^+\text{-N}$, DOC, AP, AK, and pH compared with the control (Table S3).

Plant growth

Plants in organic soils had ~56% higher total biomass than plants in control soils (Fig. 5a). In sterilized soils, plant total biomass decreased by ~65% under *N. lugens* infestation. However, when sterilized soils were inoculated with soil inoculum from organic or control treatments, plant total biomass was decreased by ~29% and ~36%, respectively, under *N. lugens* infestation. Across all treatments, the presence of soil biota significantly increased root biomass but had no effect on shoot biomass in the absence of herbivores (Fig. 5a).

Rice plants grown in organic soils had ~23% higher concentrations of shoot amino acids than plants grown in control soils in the absence of herbivores. By contrast, compared with the control, the organic treatment reduced the concentrations of shoot amino acids and sugars by up to ~41% and ~17%, respectively, under *N. lugens* infestation (Fig. 5b, c).

Plant defense traits were significantly influenced by the interaction of sterilized soil treatments and soil inocula (~18% variance explained, $p < 0.05$, Table S4b). Specifically, in the control soils, soil inoculum from the organic treatment increased the root-to-shoot ratio and stimulated the production of shoot SA compared with soil inoculum from the control treatment under *N. lugens* attack ($p < 0.05$, Fig. 5d, f). Meanwhile, shoot phenolics and SA concentrations of plants grown in control soils were increased by ~36% and ~21% in the presence of *N. lugens* infestation, respectively (Fig. 5e, f).

Planthopper abundance

Both sterilized soil and soil inoculum treatments influenced planthopper numbers at the end of the experiment (ANOVA, sterilized soil treatment effect: $F = 7.53$, $p < 0.05$; soil inoculum effect: $F = 26.12$, $p < 0.001$). Across all treatments, plants grown in organic soils had ~17% lower planthopper numbers

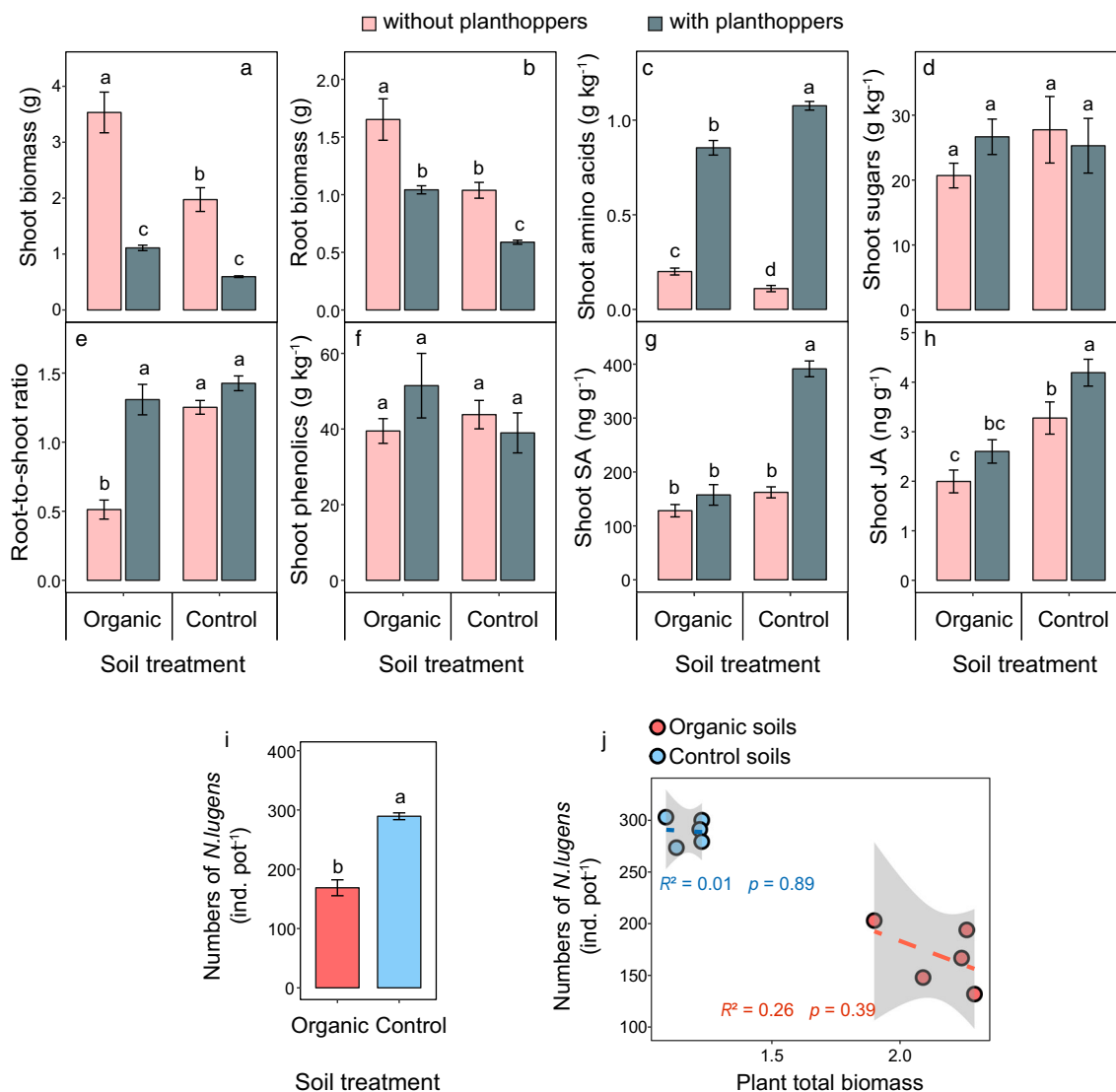


Fig. 3 Effects of organic amendments and planthopper presence on plant performance including **a** shoot biomass, **b** root biomass, **c** shoot amino acid concentration, **d** shoot sugar concentration, **e** root-to-shoot ratio, **f** shoot phenolic concentration, **g** shoot salicylic acid (SA) concentration, and **h** shoot jasmonic acid (JA) concentration. **i** Effects of organic

amendments on planthopper numbers. Bars represent standard error of the mean ($n = 5$). Different letters above bars indicate significant differences among treatments (Fisher's LSD test, $p < 0.05$). **j** Relationships between planthopper numbers and plant total biomass across soil treatments. Shaded areas indicate 95% confidence intervals

as compared to plants grown in control soils (Fig. 6a). Soil inocula suppressed the development of *N. lugens* with the magnitude being greatest for soil inocula from the organic treatment ($p < 0.05$, Fig. 6a).

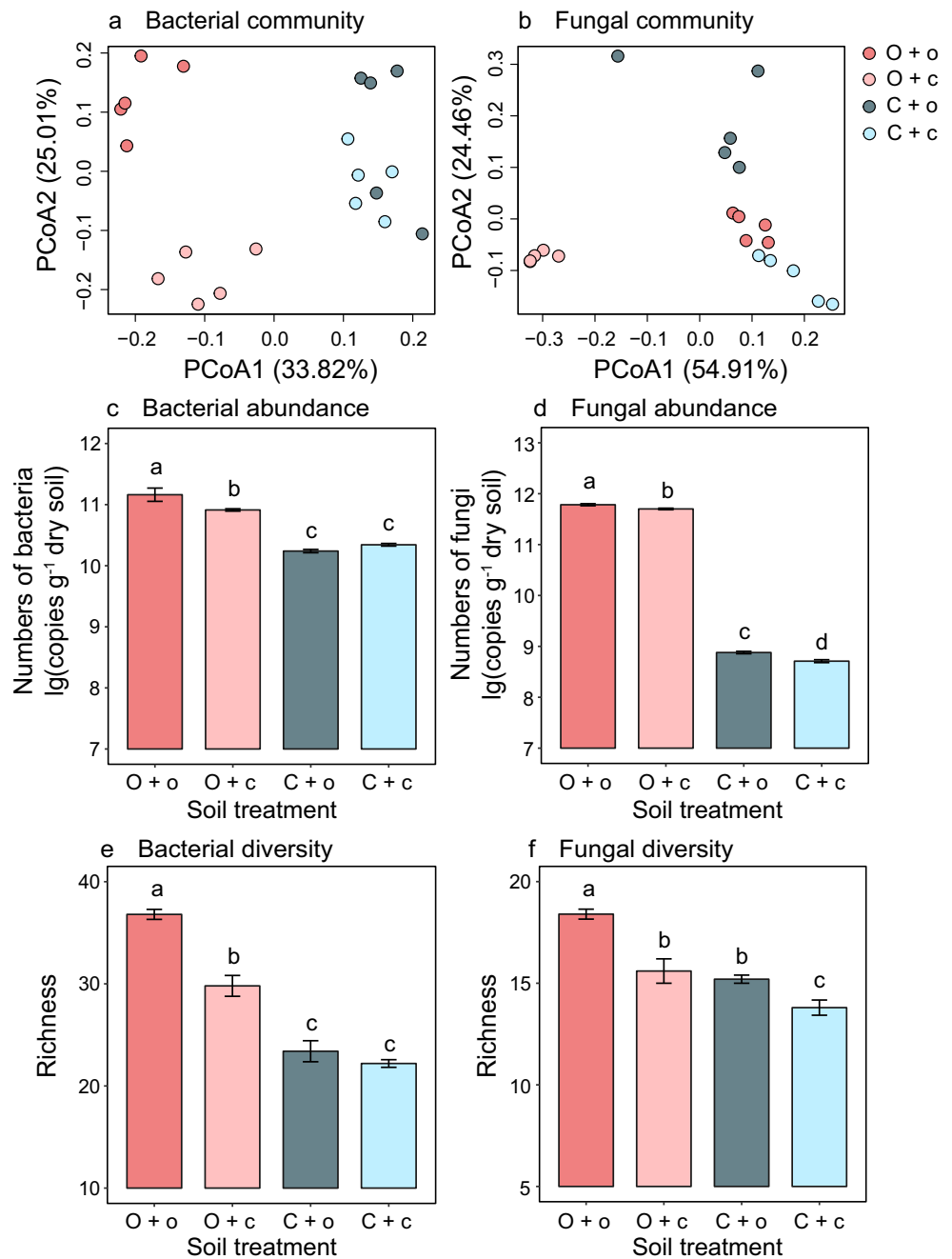
The importance of plant traits

Soil inocula explained ~42% of variation in plant biomass ($p < 0.001$, Table S4a). The random forest analysis indicated that plant total biomass was the best predictor of planthopper abundance ($p < 0.001$; Fig. 6b), and soil bacterial diversity ($p < 0.01$) was the best predictor of plant total biomass, followed by the abundance of fungi and bacteria ($p < 0.05$,

Fig. 6c). Further, planthopper abundance was negatively correlated with plant total biomass ($R^2 = 0.42$, $p < 0.01$, Fig. S1a), while positively correlated with shoot amino acid and sugar concentrations (amino acids: $R^2 = 0.24$, $p < 0.01$; sugars: $R^2 = 0.31$, $p < 0.01$, Fig. 6d; S2). In addition, plant total biomass was positively correlated with microbial abundance and diversity ($p < 0.001$, Fig. S1b), and plant nutrients (concentrations of shoot amino acids and sugars) were negatively correlated with microbial abundance and diversity ($p < 0.001$, Fig. 6e).

Plants grown in organic soils showed greater tolerance to herbivory than plants grown in control soils ($F = 5.6$, $p = 0.025$). Compared with sterilized soils, sterilized soils re-inoculated with soil inocula significantly increased plant

Fig. 4 Principal coordinate analysis (PCoA) of **a** bacterial communities and **b** fungal communities (based on T-RFLP analyses), abundance of **c** bacteria and **d** fungi (based on qPCR analyses), and diversity of **e** bacteria and **f** fungi (based on T-RFLP analyses) as influenced by different soil treatments. The experiment involved different combinations of sterilized soils and soil inocula from organic (O) or control (C) treatments. O + o and O + c, organic sterilized soils (O) with soil inoculum from the organic treatment (o) or the control treatment (c); C + o and C + c, control sterilized soils (C) with soil inoculum from the organic treatment (o) or the control treatment (c). Bars represent standard error of the mean ($n = 5$). Different letters above bars indicate significant differences among treatments (Fisher's LSD test, $p < 0.05$). Note: Sterilized soil treatments without soil inoculum are not shown



tolerance to herbivory ($p < 0.05$, Fig. 7a). Planthopper numbers negatively correlated with plant total biomass in organic soils, but did not correlate with plant total biomass in control soils (Fig. 7b).

Discussion

The aim of the study was to investigate the legacy effects of organic amendments on rice plant growth and susceptibility to aboveground herbivores, and to further disentangle effects of

biotic and abiotic soil properties as influenced by fertilization regimes on plant and herbivore performance.

Effects of soil biota on plant tolerance

Consistent with our first hypothesis, rice plant biomass was higher in organic soils than in control soils, possibly due to increased soil nutrient availability and soil biodiversity associated with organic amendments (Luo et al. 2018; Mäder et al. 2002). This suggests that reduced planthopper numbers observed in those treatments were not caused by poor vigor or

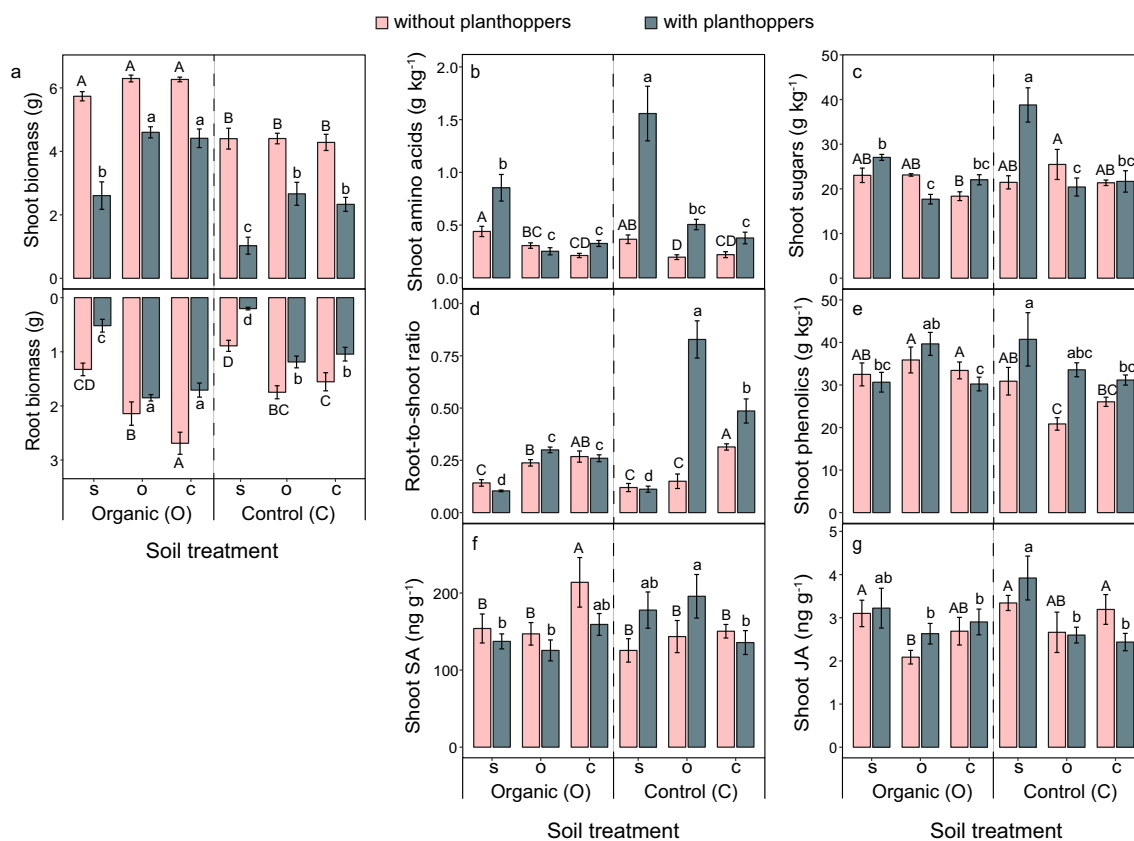


Fig. 5 Effects of sterilized soils and soil inoculum from organic (O) or control (C) treatments, and planthopper presence on plant performance including **a** plant (shoot and root) biomass, **b** shoot amino acid concentration, **c** shoot sugar concentration, **d** root-to-shoot ratio, **e** shoot phenolic concentration, **f** shoot salicylic acid (SA) concentration, and **g** shoot jasmonic acid (JA) concentration. s, sterilized soil without inoculum; o,

sterilized soil with soil inoculum from the organic treatment; c, sterilized soil with soil inoculum from the control treatment. Bars represent standard error of the mean ($n = 5$). Different capital letters above bars indicate significant differences among treatments without planthoppers and different lowercase letters indicate significant differences among treatments with planthoppers (Fisher's LSD test, $p < 0.05$)

smaller size of rice plant. At the end of the conditioning phase of experiment II, organic soils had higher nutrient availability and pH than control soils. This is in line with previous studies showing that organic amendments improved soil properties through increases in soil organic C, cation exchange capacity, and pH (Agegnehu et al. 2016; Gravuer et al. 2019; Ji et al. 2020). Generally, high nutrient availability and favorable pH could promote soil community development (Chen et al. 2019; Hu et al. 2014; Rousk et al. 2010; Tian et al. 2017), which could be the reason for the significant increment of microbial abundance and diversity in organic soils in our study.

In support of our second hypothesis, the presence of soil biota promoted plants to accumulate more biomass under planthopper attack (Fig. 5a). Soil biota may positively affect root development and the availability of nutrients for plant uptake (Richardson et al. 2009; van der Heijden et al. 2015; Verbon and Liberman 2016). Hence, the accumulation of root biomass, together with improved plant nutrition, synergistically boost plant compensatory growth (Spence et al. 2014; Van der Ent et al. 2009). Furthermore, plant growth was more affected by the abundance and diversity of their associated

soil microbial communities than by abiotic soil properties (Fig. 6c, Table S4a). This indicates that high abundance and diversity of soil biota were of pivotal importance for regulating soil nutrient availability and promoting plant growth. Similarly, earlier studies showed that high soil biodiversity facilitated the regrowth of plant tissues after herbivory by improving nutrient and water uptake (Kula et al. 2005; Saleem et al. 2019; Wagg et al. 2014), thereby promoting plant tolerance to herbivores. Taken together, our findings suggest that soil community development amended by organic amendments could promote plant growth and tolerance against herbivores via improving soil nutrient availability in agroecosystems.

Effects of soil biota on plant herbivore resistance

In further support of our first hypothesis, the concentrations of shoot amino acids in the presence of *N. lugens* were lower when rice plants were grown in organic soils than when grown in control soils. Also, substantially lower *N. lugens* numbers were detected in the treatments with organic soils. Plant

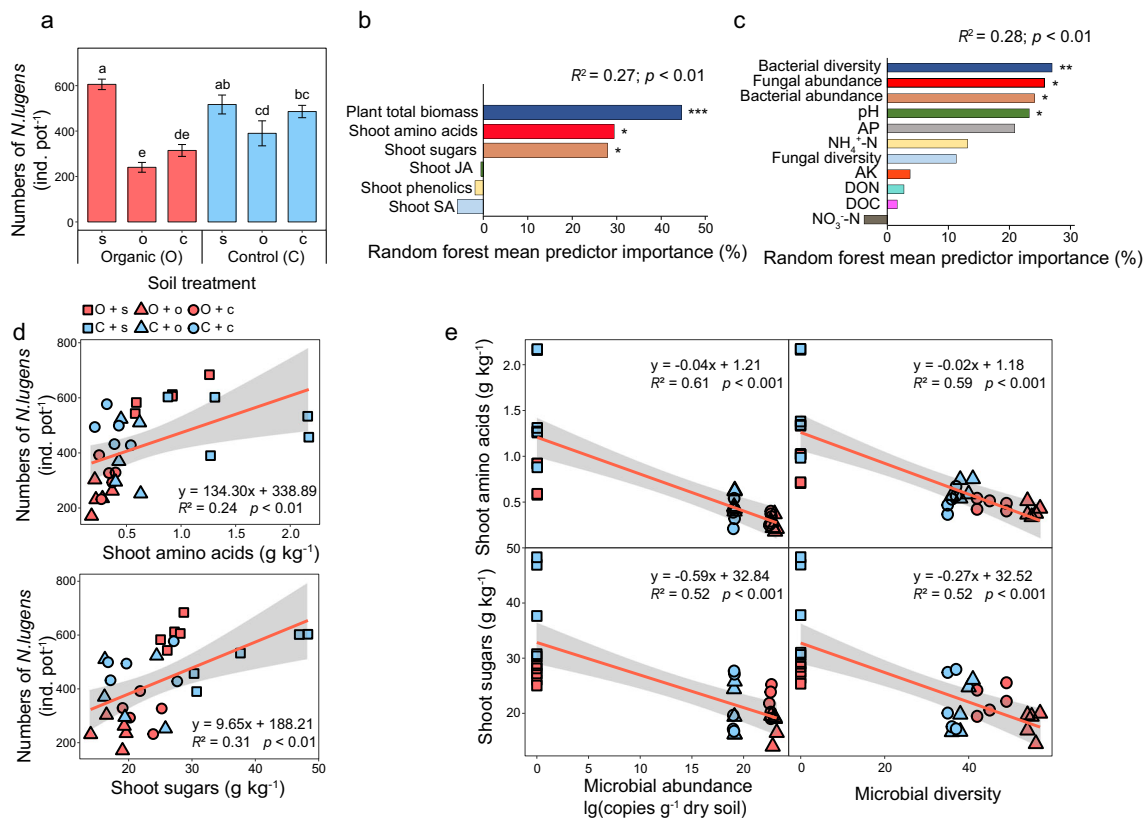


Fig. 6 **a** Effects of sterilized soils and soil inoculum from organic (O) or control (C) treatments on planthopper numbers. s, sterilized soil without inoculum; o, sterilized soil with soil inoculum from the organic treatment; c, sterilized soil with soil inoculum from the control treatment. Bars represent standard error of the mean ($n = 5$). Different letters at each bar indicate significant differences among treatments (Fisher's LSD test, $p < 0.05$). Random forest mean predictor importance (% increase in MSE) of **b** plant traits (total biomass and the concentrations of amino acids, sugars, phenolics, SA, and JA) with respect to *N. lugens* abundance and **c** abiotic soil properties (soil pH, exchangeable NH₄⁺-N, NO₃⁻-N,

DON, DOC, AP, and AK) and microbial properties (bacterial and fungal abundance and diversity) with respect to plant total biomass across treatments. SA salicylic acid, JA jasmonic acid, AP available P, AK available K, DOC dissolved organic C, DON dissolved organic N. Significance levels of each predictor are as follows: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. Relationships **d** between planthopper numbers and plant nutrients (concentrations of shoot amino acids and sugars) and **e** between plant nutrients and microbial properties (bacterial and fungal abundance and diversity) across soil treatments. Shaded areas indicate 95% confidence intervals

primary metabolites, such as amino acids and sugars, could positively affect the preference of herbivorous insects, and have been considered as a major determinant of plant resistance against herbivores (Berenbaum 1995). Therefore, lower herbivore abundance in the organic treatment might be due to reduced accumulation of plant amino acids. It has long been recognized that responses of both aboveground herbivores and plant primary metabolites are strongly positively correlated with high soil nutrient availability (Altieri and Nicholls 2003; Wu et al. 2017). However, herbivore survival can also decline with increasing availability of soil nutrients, because of increased plant production of N-based alkaloids which are toxic to herbivores (Lehtonen et al. 2005; Vesterlund et al. 2011). Both amino acids and N-based alkaloids are N-containing compounds. Hence, we cannot rule out that the high nutrient availability in organic soils in our study promoted also plant investment in alkaloids. Unraveling the mechanisms warrants further study, but our study indicates that organic amendments have a negative effect on aboveground

herbivore survival by reducing the concentrations of plant amino acids.

Interestingly, we found that soil inocula decreased plant nutrient concentrations (i.e., amino acids and sugars) in the presence of *N. lugens*, indicating that soil biota decreased plant phloem sap quality for planthoppers which strongly depend on amino acids and sugars in their diet (Awmack and Leather 2002; Badri et al. 2013; Schoonhoven et al. 2005). Notably, our reciprocal transplant experiment showed negative correlations between plant nutrients and microbial abundance and diversity (Fig. 6e). These negative correlations might be caused by plant-microbe competition for resources (Hodge et al. 2000a, b). However, several alternative mechanisms have been put forward to explain how soil biota may decrease plant nutrients in response to herbivory. For example, abundant and diverse communities of soil biota could reduce plant water stress and increase the availability of micronutrients for plants, thus facilitating protein synthesis and decrease soluble N in foliage, in turn making plants less

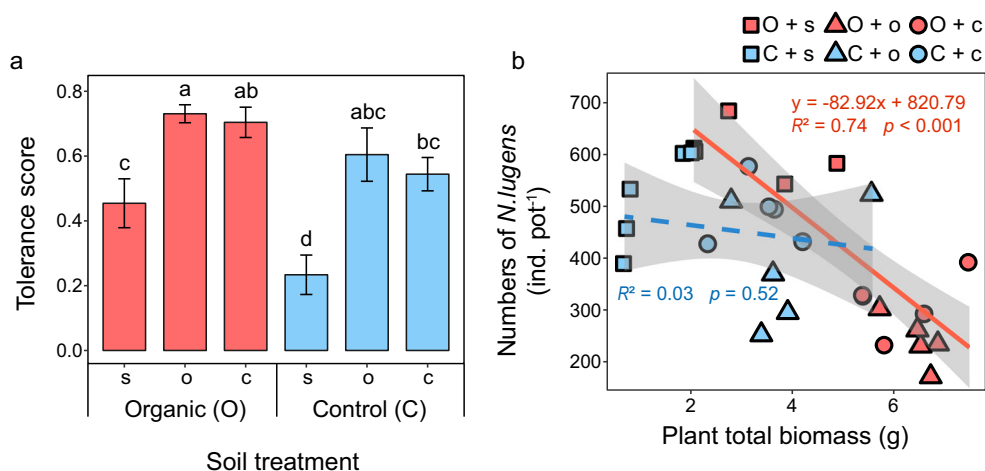


Fig. 7 a Effects of sterilized soils and soil inoculum from organic (O) or control (C) treatments on plant tolerance scores as responses to herbivores. Tolerance scores were calculated as the ratio of the shoot biomass of damaged plants relative to the mean value of undamaged plants under the same treatment conditions. s, sterilized soil without inoculum; o, sterilized soil with soil inoculum from the organic treatment; c, sterilized

soil with soil inoculum from the control treatment. Bars represent standard error of the mean ($n = 5$). Different letters at each bar indicate significant differences among treatments (Fisher's LSD test, $p < 0.05$). b Relationships between planthopper numbers and plant total biomass across soil treatments. Shaded areas indicate 95% confidence intervals

Herbivore abundance

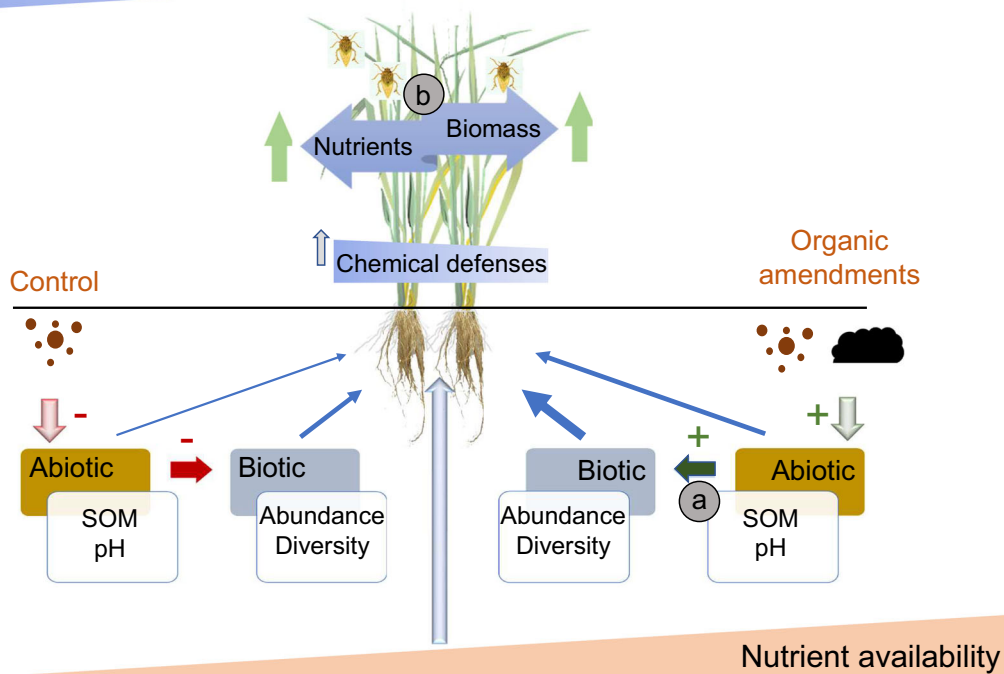


Fig. 8 A conceptual model illustrating how biotic and abiotic soil properties influenced by organic amendments affect rice growth and susceptibility to planthoppers, and planthopper performance. Compared with the control, organic amendments could support a more diverse and active soil community (e.g., increase microbial abundance and diversity) through providing essential nutrients and creating habitats for soil organisms (a, e.g., increase biologically available soil organic matter (SOM) and pH). Increased microbial activity could maintain nutrient cycling and soil structural properties thereby leading to higher nutrient availability of organic soils. Hence, high nutrient availability and biodiversity associated with organic amendments increased plant tolerance against herbivores through promoting plant growth (b). In this context,

biotic soil properties contributed more to plant growth than soil chemical properties. Also, higher microbial abundance and diversity in organic soils resulted in lower nutrients in plants, thus increasing plant resistance against planthoppers. Although rice plants grown in control soils increased defense compounds (phenolics, SA and JA), enhanced plant nutritional quality might have offset plant chemical defenses, thus making plants more palatable to herbivores. Therefore, substantially lower planthopper abundance was detected in the treatments with organic soils due to increased plant tolerance and resistance to herbivores. The thickness of the blue arrows corresponds to the strength of the positive relationships

nutritious to herbivores (Chaboussou 2004; Waring and Cobb 1992). Moreover, high soil biodiversity may promote the biologically based buffering capacity of soil and help maintain nutrient balance in crops under planthopper attack (Phelan 1997). Furthermore, under attack of aboveground herbivores, complex soil communities could stimulate plants to produce N-based alkaloids (Wang et al. 2019b), thereby reducing plant amino acids production.

Despite the key role of plant nutrients in determining plant resistance against herbivores, changes in plant chemical defenses (phenolics compounds, SA and JA) could also contribute to reduce the reproduction and development of herbivores. Previous studies showed that sap-feeding herbivores could activate the SA-signaling pathway involved in systemic acquired resistance in plants (Bezemer and van Dam 2005). However, our results showed that plant chemical defenses were not the main predictors of planthopper abundance (Fig. 6b). We found that the interactions of biotic and abiotic soil properties significantly influenced plant defenses against herbivores, indicating that effects of soil biota on plant defenses to herbivores are important and dependent on the soil treatments. In the control soils, soil inoculum obtained from the organic treatment stimulated the synthesis of plant defense compounds compared with inocula from the control treatment (Fig. 5e, f), emphasizing the tight links between soil community composition and chemical plant defenses (Pieterse et al. 2014; Rashid and Chung 2017). In the current study, although rice plants grown in control soils increased chemical defenses under planthopper attack, enhanced plant nutritional quality in plants grown in control soils might have offset plant chemical defenses, ultimately making plants more palatable to herbivores. Taken together, these findings showed that soil communities and fertilization regimes interactively regulate plant resource allocation between growth and defense.

Notably, contrary to our third hypothesis, we showed that organic amendments promoted rice plant growth without compromising anti-herbivore defenses, but instead simultaneously increased plant growth and defense against herbivores (Fig. 7b). Our analyses indicated that this was due to the high soil microbial abundance and diversity in organic soils. In contrast, previous studies showed that the induction of chemical defenses in plants in response to herbivory often comes at the expense of plant growth as plants typically are resource-limited (Coley et al. 1985; Huot et al. 2014). Here, we acknowledge that the T-RFLP methods that we used to analyze soil microbial composition and richness has some limitation as it is sensitive to the choice of genetic markers and reference databases (Xue et al. 2019). Nevertheless, our results highlight the important interactive role of biotic and abiotic soil properties in predicting plant resource allocation patterns and stress the need of utilizing organic amendments as well as the potential of regulating soil communities to concomitantly reduce the use of pesticides for sustainable crop production.

Conclusion

We showed that the plant-mediated suppressing effects of organic amendments on aboveground herbivores were linked to the positive effects of organic amendments on soil microbial abundance and diversity. Specifically, compared with the control treatment, higher essential nutrients and pH in the organic treatment increased microbial abundance and diversity (Fig. 8a). The diverse soil biota associated with organic amendments suppressed aboveground herbivory via enhancing plant tolerance (i.e., increasing plant total biomass) and resistance as mediated by plant resource allocation of primary metabolites (i.e., decreasing concentrations of sugars and amino acids) (Fig. 8b). We further demonstrated that plant growth was more closely related to their associated soil microbial community than to soil chemical properties, while plant defenses were strongly affected by the interactions of biotic and abiotic soil factors. We therefore suggest the vital role of soil biota to be considered in predicting the allocation of plant resources to growth and defense based on the resource availability hypothesis. Taken together, our findings highlight the importance of disentangling the influences of biotic and abiotic drivers to mechanistically understand the ecosystem functioning across agricultural management practices. Nevertheless, it remains largely unknown how different soil communities or even specific compositional traits could control resource allocation patterns and plant resistance. Hence, further studies are needed, for example by combining field surveys with functional metagenome and transcriptome methodology of soils, plants, and insect herbivores.

Acknowledgments This work was funded by the Natural Science Foundation of China (41771287 and 41877056), the Jiangxi Key R&D program (20181ACH80007) and the Innovative Foreign Experts Introduction Plan for National Key Discipline of Agricultural Resources and Environment (B12009). PK acknowledges support from the Swedish Research Council (Vetenskapsrådet). We thank Nan Jin, Xin Gong and other members of Soil Ecology Lab for their help in the laboratory work and Prof. Wei Huang for helpful comments.

References

- Agegnehu G, Nelson PN, Bird MI (2016) Crop yield, plant nutrient uptake and soil physicochemical properties under organic soil amendments and nitrogen fertilization on Nitisols. *Soil Tillage Res* 160:1–13
- Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic and other oxidation substrates in plant tissues using folin-ciocalteu reagent. *Nat Protoc* 2:875–877
- Almasia R, Carú M, Handford M, Orlando J (2016) Environmental conditions shape soil bacterial community structure in a fragmented landscape. *Soil Biol Biochem* 103:39–45
- Altieri MA, Nicholls CI (2003) Soil fertility management and insect pests: harmonizing soil and plant health in agroecosystems. *Soil Tillage Res* 72:203–211

- Alyokhin A, Porter G, Groden E, Drummond F (2005) Colorado potato beetle response to soil amendments: a case in support of the mineral balance hypothesis? *Agric Ecosyst Environ* 109:234–244
- Archer E (2016) Estimate permutation p-values for importance metrics. R Package Version 2.0
- Awmack CS, Leather SR (2002) Host plant quality and fecundity in herbivorous insects. *Annu Rev Entomol* 47:817–844
- Badri DV, Zolla G, Bakker MG, Manter DK, Vivanco JM (2013) Potential impact of soil microbiomes on the leaf metabolome and on herbivore feeding behavior. *New Phytol* 198:264–273
- Bardgett RD, van der Putten WH (2014) Belowground biodiversity and ecosystem functioning. *Nature* 515:505–511
- Bechtold U, Ferguson JN, Mullineaux PM (2018) To defend or to grow: lessons from *Arabidopsis* C24. *J Exp Bot* 69:2809–2821
- Bedada W, Karlun E, Lemenih M, Tolera M (2014) Long-term addition of compost and NP fertilizer increases crop yield and improves soil quality in experiments on smallholder farms. *Agric Ecosyst Environ* 195:193–201
- Bender SF, Wagg C, van der Heijden MG (2016) An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends Ecol Evol* 31:440–452
- Berenbaum MR (1995) Turnabout is fair play: secondary roles for primary compounds. *J Chem Ecol* 21:925–940
- Bezemer TM, van Dam NM (2005) Linking aboveground and belowground interactions via induced plant defenses. *Trends Ecol Evol* 20:617–624
- Bottrell DG, Schoenly KG (2012) Resurrecting the ghost of green revolutions past: the brown planthopper as a recurring threat to high-yielding rice production in tropical Asia. *J Asia Pac Entomol* 15:122–140
- Breiman L (2001) Random forests. *Mach Learn* 45:5–32
- Cahill JF, Cale JA, Karst J, Bao T, Pec GJ, Erbilgin N (2017) No silver bullet: different soil handling techniques are useful for different research questions, exhibit differential type I and II error rates, and are sensitive to sampling intensity. *New Phytol* 216:11–14
- Chaboussou F (2004) Healthy crops: a new agricultural revolution. Jon Carpenter Publishing, Oxford
- Chen J, Chen D, Xu Q, Fuhrmann JJ, Li L, Pan G, Li Y, Qin H, Liang C, Sun X (2019) Organic carbon quality, composition of main microbial groups, enzyme activities, and temperature sensitivity of soil respiration of an acid paddy soil treated with biochar. *Biol Fertil Soils* 55:185–197
- Coley PD, Bryant JP, Chapin FS (1985) Resource availability and plant antiherbivore defense. *Science* 230:895–899
- D'Costa L, Simmonds MSJ, Straw N, Castagneyrol B, Koricheva J (2014) Leaf traits influencing oviposition preference and larval performance of *Cameraria ohridella* on native and novel host plants. *Entomol Exp Appl* 152:157–164
- Delgado-Baquerizo M, Gallardo A, Covelo F, Prado-Comesaña A, Ochoa V, Maestre FT (2015) Differences in thallus chemistry are related to species-specific effects of biocrust-forming lichens on soil nutrients and microbial communities. *Funct Ecol* 29:1087–1098
- Delgado-Baquerizo M, Trivedi P, Trivedi C, Eldridge DJ, Reich PB, Jeffries TC, Singh BK (2017) Microbial richness and composition independently drive soil multifunctionality. *Funct Ecol* 31:2330–2343
- Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, Mueller ND, O'Connell C, Ray DK, West PC, Balzer C, Menent EM, Carpenter SR, Hill J, Monfreda C, Polasky S, Rockström J, Sheehan J, Siebert S, Tilman D, Zaks DPM (2011) Solutions for a cultivated planet. *Nature* 478:337–342
- Fortmann-Roe S (2013) Accurate, adaptable, and accessible error metrics for predictive. R package version 0.9.2
- Friessen ML, Porter SS, Stark SC, Von Wettberg EJ, Sachs JL, Martinez-Romero E (2011) Microbially mediated plant functional traits. *Annu Rev Ecol Evol Syst* 42:23–46
- Glassman SI, Weihe C, Li J, Albright MBN, Looby CI, Martiny AC, Treseder KK, Allison SD, Martiny JBH (2018) Decomposition responses to climate depend on microbial community composition. *PNAS* 115:11994–11999
- Gong X, Wang S, Wang Z, Jiang Y, Hu Z, Zheng Y, Chen X, Li H, Hu F, Liu M, Scheu S (2019) Earthworms modify soil bacterial and fungal communities through enhancing aggregation and buffering pH. *Geoderma* 347:59–69
- Gravuer K, Gennet S, Throop HL (2019) Organic amendment additions to rangelands: a meta-analysis of multiple ecosystem outcomes. *Glob Chang Biol* 25:1152–1170
- Griffiths BS, Ritz K, Wheatley R, Kuan HL, Boag B, Christensen S, Ekelund F, Sørensen SJ, Muller S, Bloem J (2001) An examination of the biodiversity-ecosystem function relationship in arable soil microbial communities. *Soil Biol Biochem* 33:1713–1722
- Grime JP, Brown VK, Thompson K, Masters GJ, Hillier SH, Clarke IP, Askew AP, Corker D, Kieley JP (2000) The response of two contrasting limestone grasslands to simulated climate change. *Science* 289:762–765
- Gundale MJ, Wardle DA, Kardol P, van der Putten WH, Lucas RW (2017) Soil handling methods should be selected based on research questions and goals. *New Phytol* 216:18–23
- Heinen R, Biere A, Harvey JA, Bezemer TM (2018) Effects of soil organisms on aboveground plant-insect interactions in the field: patterns, mechanisms and the role of methodology. *Front Ecol Evol* 6:106
- Heinen R, Biere A, Bezemer TM (2020) Plant traits shape soil legacy effects on individual plant-insect interactions. *Oikos* 129:261–273
- Hettenhausen C, Baldwin IT, Wu J (2013) *Nicotiana attenuata* MPK4 suppresses a novel jasmonic acid (JA) signaling-independent defense pathway against the specialist insect *Manduca sexta*, but is not required for the resistance to the generalist *Spodoptera littoralis*. *New Phytol* 199:787–799
- Hodge A, Stewart J, Robinson D, Griffiths BS, Fitter AH (2000a) Competition between roots and soil micro-organisms for nutrients from nitrogen-rich patches of varying complexity. *J Ecol* 88:150–164
- Hodge A, Robinson D, Fitter A (2000b) Are microorganisms more effective than plants at competing for nitrogen? *Trends Plant Sci* 5:304–308
- Hu Y, Xiang D, Veresoglou SD, Chen F, Chen Y, Hao Z, Zhang X, Chen B (2014) Soil organic carbon and soil structure are driving microbial abundance and community composition across the arid and semi-arid grasslands in northern China. *Soil Biol Biochem* 77:51–57
- Huang Q, Hu F, Huang S, Li H, Yuan Y, Pan G, Zhang W (2009) Effect of long-term fertilization on organic carbon and nitrogen in a subtropical paddy soil. *Pedosphere* 19:727–734
- Huang W, Siemann E, Wheeler GS, Zou J, Carrillo J, Ding J (2010) Resource allocation to defence and growth are driven by different responses to generalist and specialist herbivory in an invasive plant. *J Ecol* 98:1157–1167
- Huang J, Liu M, Chen F, Griffiths BS, Chen X, Johnson SN, Hu F (2012) Crop resistance traits modify the effects of an aboveground herbivore, brown planthopper, on soil microbial biomass and nematode community via changes to plant performance. *Soil Biol Biochem* 49:157–166
- Huang J, Liu M, Chen X, Chen J, Chen F, Li H, Hu F (2013) Intermediate herbivory intensity of an aboveground pest promotes soil labile resources and microbial biomass via modifying rice growth. *Plant Soil* 367:437–447
- Huot B, Yao J, Montgomery BL, He SY (2014) Growth-defense tradeoffs in plants: a balancing act to optimize fitness. *Mol Plant* 7:1267–1287
- Ialongo C (2016) Understanding the effect size and its measures. *Biochem Med* 26:150–163
- Ikoyi I, Egeter B, Chaves C, Ahmed M, Fowler A, Schmalenberger A (2020) Responses of soil microbiota and nematodes to application of

- organic and inorganic fertilizers in grassland columns. *Biol Fertl Soils*. <https://doi.org/10.1007/s00374-020-01440-5>
- IUSS Working Group WRB (2006) World reference base for soil resources. World Soil Resources Report 103. FAO Rome, Italy
- Ji L, Ni K, Wu Z, Zhang J, Yi X, Yang X, Ling N, You Z, Guo S, Ruan J (2020) Effect of organic substitution rates on soil quality and fungal community composition in a tea plantation with long-term fertilization. *Biol Fertl Soils*. <https://doi.org/10.1007/s00374-020-01439-y>
- Karban R, Baldwin IT (1997) Induced responses to herbivory. University of Chicago Press, Chicago
- Knapp S, van der Heijden MGA (2018) A global meta-analysis of yield stability in organic and conservation agriculture. *Nat Commun* 9: 3632
- Kula AAR, Hartnett DC, Wilson GWT (2005) Effects of mycorrhizal symbiosis on tallgrass prairie plant-herbivore interactions. *Ecol Lett* 8:61–69
- Lau JA, Lennon JT (2012) Rapid responses of soil microorganisms improve plant fitness in novel environments. *PNAS* 109:14058–14062
- Lehtonen P, Helander M, Saikkonen K (2005) Are endophyte-mediated effects on herbivores conditional on soil nutrients? *Oecologia* 142: 38–45
- Liaw A, Wiener M (2002) Classification and regression by randomForest. *R News* 2:18–22
- Liu M, Hu F, Chen X, Huang Q, Jiao J, Zhang B, Li H (2009) Organic amendments with reduced chemical fertilizer promote soil microbial development and nutrient availability in a subtropical paddy field: the influence of quantity, type and application time of organic amendments. *Appl Soil Ecol* 42:166–175
- Liu T, Yang L, Hu Z, Xue J, Lu Y, Chen X, Griffiths BS, Whalen JK, Liu M (2020) Biochar exerts negative effects on soil fauna across multiple trophic levels in a cultivated acidic soil. *Biol Fertl Soils*. <https://doi.org/10.1007/s00374-020-01436-1>
- Lozupone C, Hamady M, Knight R (2006) UniFrac—an online tool for comparing microbial community diversity in a phylogenetic context. *BMC Bioinformatics* 7:371
- Luo G, Li L, Friman V, Guo J, Guo S, Shen Q, Ling N (2018) Organic amendments increase crop yields by improving microbe-mediated soil functioning of agroecosystems: a meta-analysis. *Soil Biol Biochem* 124:105–115
- Ma C, Liu M, Wang H, Chen C, Fan W, Griffiths BS, Li H (2015) Resource utilization capability of bacteria predicts their invasion potential in soil. *Soil Biol Biochem* 81:287–290
- Ma H, Pineda A, Hannula SE, Kielak AM, Setyarini SN, Bezemer TM (2020) Steering root microbiomes of a commercial horticultural crop with plant-soil feedbacks. *Appl Soil Ecol* 150:103468
- Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P, Niggli U (2002) Soil fertility and biodiversity in organic farming. *Science* 296:1694–1697
- Mariotte P, Mehrabi Z, Bezemer TM, De Deyn GB, Kulmatiski A, Drigo B, Veen GF, van der Heijden MGA, Kardol P (2018) Plant-soil feedback: bridging natural and agricultural sciences. *Trends Ecol Evol* 33:129–142
- Martinez-Medina A, Flors V, Heil M, Mauch-Mani B, Pieterse CM, Pozo MJ, Ton J, van Dam NM, Conrath U (2016) Recognizing plant defense priming. *Trends Plant Sci* 21:818–822
- Masuko T, Minami A, Iwasaki N, Majima T, Lee YC (2005) Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Anal Biochem* 339:69–72
- Muller A, Schader C, Scialabba NEH, Brüggemann J, Isensee A, Erb KH, Smith P, Klocke P, Leiber F, Stolze M, Niggli U (2017) Strategies for feeding the world more sustainably with organic agriculture. *Nat Commun* 8:1290
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2018) Vegan: community ecology package. R Package Version 2.5–3
- Pan X, Welti R, Wang X (2010) Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography-mass spectrometry. *Nat Protoc* 5:986–992
- Phelan PL (1997) Soil-management history and the role of plant mineral balance as a determinant of maize susceptibility to the European corn borer. *Biol Agric Hortic* 15:25–34
- Phelan PL, Norris KH, Mason JF (1996) Soil-management history and host preference by *Ostrinia nubilalis*: evidence for plant mineral balance mediating insect-plant interactions. *Environ Entomol* 25: 1329–1336
- Pieterse CMJ, Zamioudis C, Berendsen RL, Weller DM, Van Wees SCM, Bakker PAHM (2014) Induced systemic resistance by beneficial microbes. *Annu Rev Phytopathol* 52:347–375
- Pineda A, Zheng S, van Loon JJA, Pieterse CMJ, Dicke M (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. *Trends Plant Sci* 15:507–514
- Pineda A, Dicke M, Pieterse CMJ, Pozo MJ (2013) Beneficial microbes in a changing environment: are they always helping plants to deal with insects? *Funct Ecol* 27:574–586
- Pineda A, Kaplan I, Bezemer TM (2017) Steering soil microbiomes to suppress aboveground insect pests. *Trends Plant Sci* 22:770–778
- Pineda A, Kaplan I, Hannula SE, Ghanem W, Bezemer TM (2020) Conditioning the soil microbiome through plant-soil feedbacks suppresses an aboveground insect pest. *New Phytol* 226:595–608
- Puga-Freitas R, Blouin M (2015) A review of the effects of soil organisms on plant hormone signalling pathways. *Environ Exp Bot* 114:104–116
- Qiao C, Penton CR, Xiong W, Liu C, Wang R, Liu Z, Xu X, Li R, Shen Q (2019) Reshaping the rhizosphere microbiome by bio-organic amendment to enhance crop yield in a maize-cabbage rotation system. *Appl Soil Ecol* 142:136–146
- Raaijmakers JM, Mazzola M (2015) Soil immune responses. *Science* 352:1392–1393
- Rashid MH, Chung YR (2017) Induction of systemic resistance against insect herbivores in plants by beneficial soil microbes. *Front Plant Sci* 8:1816–1826
- Reganold JP, Wachter JM (2016) Organic agriculture in the twenty-first century. *Nat Plants* 2:15221
- Reinhart KO, Rinella MJ (2016) A common soil handling technique can generate incorrect estimates of soil biota effects on plants. *New Phytol* 210:786–789
- Richardson AE, Barea J, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil* 321:305–339
- Rodríguez-Echeverría S, Armas C, Pistón N, Hortal S, Pugnaire FI, De Deyn G (2013) A role for below-ground biota in plant-plant facilitation. *J Ecol* 101:1420–1428
- Rohsius C, Matissek R, Lieberei R (2006) Free amino acid amounts in raw cocoas from different origins. *Eur Food Res Technol* 222:432–438
- Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J* 4:1340–1351
- Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Global biodiversity scenarios for the year 2100. *Science* 287:1770–1774
- Saleem M, Hu J, Jousset A (2019) More than the sum of its parts: microbiome biodiversity as a driver of plant growth and soil health. *Annu Rev Ecol Syst* 50:145–168
- Schoonhoven LM, van Loon Joop JA, Dicke M (2005) Insect-plant biology. Oxford University Press, Oxford
- Shi W, Ju Y, Bian R, Li L, Joseph S, Mitchell DR, Munroe P, Taherymoosavi S, Pan G (2020) Biochar bound urea boosts plant

- growth and reduces nitrogen leaching. *Sci Total Environ* 701: 134424
- Simms EL, Triplett J (1994) Costs and benefits of plant responses to disease: resistance and tolerance. *Evolution* 48:1973–1985
- Singh JS, Gupta VK (2018) Soil microbial biomass a key soil driver in management of ecosystem functioning. *Sci Total Environ* 634:497–500
- Spence C, Alff E, Johnson C, Ramos C, Donofrio N, Sundaresan V, Bais H (2014) Natural rice rhizospheric microbes suppress rice blast infections. *BMC Plant Biol* 14:130–146
- Strauss SY, Agrawal AA (1999) The ecology and evolution of plant tolerance to herbivory. *Trends Ecol Evol* 14:179–185
- Teste FP, Kardol P, Turner BL, Wardle DA, Zemunik G, Renton M, Laliberté E (2019) Toward more robust plant-soil feedback research: comment. *Ecology* 100:e02590
- Tian J, Lou Y, Gao Y, Fang H, Liu S, Xu M, Blagodatskaya E, Kuzyakov Y (2017) Response of soil organic matter fractions and composition of microbial community to long-term organic and mineral fertilization. *Biol Fertil Soils* 53:523–532
- Trivedi C, Delgado-Baquerizo M, Hamonts K, Lai K, Reich PB, Singh BK (2019) Losses in microbial functional diversity reduce the rate of key soil processes. *Soil Biol Biochem* 135:267–274
- van de Voorde TFJ, van der Putten WH, Bezemer TM (2012) Soil inoculation method determines the strength of plant-soil interactions. *Soil Biol Biochem* 55:1–6
- Van der Ent S, Van Wees SCM, Pieterse CMJ (2009) Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. *Phytochemistry* 70:1581–1588
- van der Heijden MGA, de Bruin S, Luckerhoff L, van Logtestijn RSP, Schlaeppi K (2015) A widespread plant-fungal-bacterial symbiosis promotes plant biodiversity, plant nutrition and seedling recruitment. *ISME J* 10:389–399
- Verbon EH, Liberman LM (2016) Beneficial microbes affect endogenous mechanisms controlling root development. *Trends Plant Sci* 21: 218–229
- Vesterlund SR, Helander M, Faeth SH, Hyvonen T, Saikkonen K (2011) Environmental conditions and host plant origin override endophyte effects on invertebrate communities. *Fungal Divers* 47:109–118
- Wagg C, Bender SF, Widmer F, van der Heijden MGA (2014) Soil biodiversity and soil community composition determine ecosystem multifunctionality. *PNAS* 111:5266–5270
- Wang G, Govinden R, Chenia HY, Ma Y, Guo D, Ren G (2019a) Suppression of *Phytophthora* blight of pepper by biochar amendment is associated with improved soil bacterial properties. *Biol Fertil Soils* 55:813–824
- Wang M, Ruan W, Kostenko O, Carvalho S, Hannula SE, Mulder PPJ, Bu F, van der Putten WH, Bezemer TM (2019b) Removal of soil biota alters soil feedback effects on plant growth and defense chemistry. *New Phytol* 221:1478–1491
- Waring GL, Cobb NS (1992) The impact of plant stress on herbivore population dynamics. In: Bems EA (ed) *Insect-plant interactions*. CRC Press, Boca Raton, pp 167–226
- Wu X, Yu Y, Baerson SR, Song Y, Liang G, Ding C, Niu J, Pan Z, Zeng R (2017) Interactions between nitrogen and silicon in rice and their effects on resistance toward the brown Planthopper *Nilaparvata lugens*. *Front Plant Sci* 8:28
- Xiao Z, Liu M, Jiang L, Chen X, Griffiths BS, Li H, Hu F (2016) Vermicompost increases defense against root-knot nematode (*Meloidogyne incognita*) in tomato plants. *Appl Soil Ecol* 105: 177–186
- Xiao Z, Jiang L, Chen X, Zhang Y, Defossez E, Hu F, Liu M, Rasmann S (2019) Earthworms suppress thrips attack on tomato plants by concomitantly modulating soil properties and plant chemistry. *Soil Biol Biochem* 130:23–32
- Xu Y, Ge Y, Song J, Rensing C (2020) Assembly of root-associated microbial community of typical rice cultivars in different soil types. *Biol Fertil Soils* 56:249–260
- Xue C, Hao Y, Pu X, Penton CR, Wang Q, Zhao M, Zhang B, Ran W, Huang Q, Shen Q, Tiedje JM (2019) Effect of LSU and ITS genetic markers and reference databases on analyses of fungal communities. *Biol Fertil Soils* 55:79–88
- Yadav DS, Chander S (2010) Simulation of rice planthopper damage for developing pest management decision support tools. *Crop Prot* 29: 267–276
- Zhu F, Heinen R, van der Sluijs M, Raaijmakers C, Biere A, Bezemer TM (2018) Species-specific plant-soil feedbacks alter herbivore-induced gene expression and defense chemistry in *Plantago lanceolata*. *Oecologia* 188:801–811
- Züst Y, Agrawal AA (2017) Trade-offs between plant growth and defense against insect herbivory: an emerging mechanistic synthesis. *Annu Rev Plant Biol* 68:513–534

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.