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Soil biota, carbon cycling and crop plant biomass responses to biochar in a temperate mesocosm experiment

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Abstract

Background and aims

Biochar addition to soil is a carbon capture and storage option with potential to mitigate rising atmospheric CO_2 concentrations, yet the consequences for soil organisms and linked ecosystem processes are inconsistent or unknown. We tested biochar impact on soil biodiversity, ecosystem functions, and their interactions, in temperate agricultural soils.

Methods

We performed a 27-month factorial experiment to determine effects of biochar, soil texture, and crop species treatments on microbial biomass (PFLA), soil invertebrate density, crop biomass and ecosystem CO_2 flux in plant-soil mesocosms.

Results

Overall soil microbial biomass, microarthropod abundance and crop biomass were unaffected by biochar, although there was an increase in fungal-bacterial ratio and a positive relationship between the 16:1ω5 fatty acid marker of AMF mass and collembolan density in the biochar-treated mesocosms. Ecosystem CO_2 fluxes were unaffected by biochar, but soil carbon content of biochar-treated mesocosms was significantly lower, signifying a possible movement/loss of biochar or priming effect.

Conclusions

Compared to soil texture and crop type, biochar had minimal impact on soil biota, crop production and carbon cycling. Future research should examine subtler effects of biochar on biotic regulation of ecosystem production and if the apparent robustness to biochar weakens over greater time spans or in combination with other ecological perturbations.

Introduction

Globally, the largest terrestrial stock of organic carbon is contained within the soil, a critical factor in the earth's carbon balance (Lal 2004). Soil carbon pools are predicted to diminish in response to climate change as warmer temperatures enhance microbial decomposition rates leading to feedbacks, including accelerated release of previously stable soil carbon (Gebremikael et al. 2016; Wardle et al. 2008). Consequently, there is considerable interest in ecological engineering of soils to enhance soil carbon stocks and thereby regulate soil carbon emissions to the atmosphere (Smith 2016). One such strategy is the capture of atmospheric CO_2 within biomass and subsequent production of biochar – a slow-cycling, carbon-rich substance – for storage in the soil (Lehmann 2007; Wang et al. 2016). However, the biotic complexity of belowground systems is likely to influence soil functional responses to both climate change and ecological engineering (Bardgett and van der Putten 2014; McCormack et al. 2013; Nielsen et al. 2011).

The term 'biochar' refers to a range of residues produced from the oxygen-limited pyrolysis of organic matter. Biochar has been shown to increase agricultural productivity while augmenting terrestrial organic carbon stocks (Lehmann and Rondon 2006), although this effect is often highly context-specific, varying with soil type, crop species and biome (Backer et al. 2016; Jeffery et al. 2011). However, biochar application can also affect the cycling and storage of pre-existing soil organic carbon and the organisms underpinning these processes (Wang et al. 2016). Some studies report that biochar has a mean residence time in soil of centuries and contributes to the stabilisation of pre-existing soil carbon (Liang et al. 2010; Maestrini et al. 2015; Wang et al. 2016; Zheng et al. 2018). Others, however, have found that the introduction of biochar stimulates microbial activity, which primes the loss of soil carbon (Maestrini et al. 2015; Steinbeiss et al. 2009; Wardle et al. 2008).

Rates of carbon mineralization by decomposers, such as microbes and detritivorous invertebrates, are influenced by higher trophic levels, including microbial-feeding and predatory invertebrates (Ayres et al. 2010). These higher trophic levels can exert a regulatory influence on soil carbon storage despite only directly contributing to a relatively small proportion of soil carbon mineralisation (Ayres et al. 2010). Hence, the soil's response to biochar addition can be expected to depend on the impact on soil fauna, but this relationship is not yet well understood (McCormack et al. 2013). For example, biochar-induced changes to the density of microbial-feeding invertebrates may influence soil carbon balance, *via* feeding activities that can influence the abundance and activity of decomposer populations (Staddon et al. 2003).

Although soil communities are typically characterised by high species diversity and functional redundancy (Bardgett and van der Putten 2014), land management practices and land-use changes may elicit dramatic shifts in soil faunal and microbial communities with potential consequences for soil ecosystem function (Heemsbergen et al. 2004). The response of the soil biota to biochar addition may therefore account for some of the variation in soil CO_2 fluxes observed following addition of biochar (Jeffery et al. 2011; Lehmann et al. 2011). This effect of biochar on the physical and biological nature of soils needs to be understood better to gauge its efficacy as a long-term carbon capture and storage option (McCormack et al. 2013; Steinbeiss et al. 2009).

The effects of biochar on soil communities may be driven by the physical and chemical changes it elicits in the soil habitat (Lehmann et al. 2011). Biochar properties can vary according to production conditions and feedstock; however, certain characteristics are common to most biochar types, including a neutral to alkaline pH, a low bulk density, and a relatively high resistance to microbial degradation (Sohi et al. 2009). Furthermore, biochar is typically exceptionally porous with a high surface area and cation exchange capacity (CEC), and hence

97 can improve soil retention of water, nutrients, heavy metals and organic compounds (Chan and
98 Xu 2009; Sohi et al. 2009). While some of these modifications to soil properties potentially
99 benefit crop growth, they may also cause unintended changes to the soil biota and the processes
100 they underpin (Lehmann et al. 2011; McCormack et al. 2013; Staddon et al. 2003). For instance,
101 augmented retention of soil nutrients and water could stimulate microbial activity, thereby
102 causing unintended loss of non-pyrogenic soil carbon (Staddon et al. 2003; Wardle et al. 2008).
103 Biochar can be applied in a wide range of environmental situations, including different types
104 of soil and cropping regimes, which makes predicting biochar-induced changes to biotic carbon
105 cycling challenging (McCormack et al. 2013).

106 Further complicating our understanding of biochar impacts on soil biota and functioning is that
107 biochar is composed of a labile carbon, an ash, and a stable carbon fraction, which differ in
108 potential effects on the soil ecosystem. The stable carbon fraction is usually the largest
109 proportion, although this varies with feedstock and production conditions, and is relatively inert
110 (Cross and Sohi 2011; Wang et al. 2016). The labile carbon fraction can be a substrate for
111 decomposers within the soil food web, while the ash fraction can also contain nutrients or toxic
112 organic compounds, with the potential to affect soil biodiversity (Lehmann et al. 2011;
113 McCormack et al. 2013; Steinbeiss et al. 2009). While effects of the labile and ash components
114 of biochar on the soil biota may be strong, they are often short-lived due to mineralisation and
115 leaching (Cross and Sohi 2011; Hol et al. 2017; Wang et al. 2016). To understand if biochar
116 induces sustained changes to soil function and community composition requires longer-term
117 studies spanning multiple seasons.

118 Soil microbial communities have a crucial function as decomposers that directly regulate
119 organic carbon cycling (Bardgett and van der Putten 2014) and numerous studies have
120 addressed the impacts of biochar on this aspect of functional biodiversity (Jenkins et al. 2017;
121 Wardle et al. 2008). Biochar often stimulates microbial abundance and activity (Lehmann et

al. 2011) and has been found to promote bacterial over fungal decomposition pathways by reducing soil acidity and increasing soil nutrient availability (Chen et al. 2013; Prayogo et al. 2014). Such changes to primary decomposers are likely to affect higher trophic levels in the food web and potentially feedback in complex ways to modify soil nutrient cycling (McCormack et al. 2013). These impacts on the soil community may therefore have implications for soil fertility, plant productivity and soil carbon storage (Domene et al. 2015; Lehmann et al. 2011). While there is a clear capacity for biochar additions to affect soil properties and biotic communities with feedbacks to ecosystem carbon cycling, there is a paucity of experimental data that can disentangle interactions between biochar addition, soil type and land use on soil biodiversity and function (Domene et al. 2015; McCormack et al. 2013).

The goal of this study was to test how biochar impacts a range of functionally important soil biodiversity (microbes, nematodes, collembola, mites) and ecosystem functions, namely CO_2 fluxes and crop plant production. This was done using a three-year factorial experiment where we manipulated biochar presence, crop plant species and soil type to mimic, in different agricultural contexts, the impact of biochar on soil biota and ecosystem function. Specifically, we made the following predictions:

i. Biochar would lower the ratio of fungi to bacteria by increasing soil water holding capacity, labile carbon content, and soil pH.
ii. Biochar-induced changes to soil properties and reductions in fungal biomass would modify invertebrate communities, indicated by differential shifts in nematode and microarthropod (mites, collembola) abundance.
iii. The effects of biochar addition on soil chemical, physical and biological properties would augment plant productivity, and increase rates of ecosystem carbon uptake and mineralisation.

Materials and Methods

Experimental design

The experiment was established at the Centre for Ecology and Hydrology in Penicuik, UK (55° 51’ N, 3° 12’ W, altitude 189 m) in a fenced, outdoor enclosure (Fig. 1S). The fully-factorial experimental design comprised three treatments: 1) biochar (absence or presence at 2 % w/w); 2) plant type (barley, perennial ryegrass, or unvegetated); and 3) soil texture (sandy clay, sandy silt loam, clay loam). Four replicates of each treatment combination (18 combinations in total) were produced. Mesocosms (72 in total) were randomly positioned into four adjacent spatial blocks, with one replicate of each treatment combination per block. All soils were obtained from the top 20 cm of the soil profile, from the James Hutton Institute’s Balruderry Farm near Dundee, in the east of Scotland, UK (56° 27’ N, 3° 4’ W, National Grid Reference NO304329, 29 m above sea level) in April, 2011. The underlying parent material of the soils was raised beach sand/gravel derived mainly from Old Red Sandstone sediments. The soils were Brown Forest Soils of the Balrownie and Garvock series (Soil Survey for Scotland nomenclature: https://soils.environment.gov.scot/maps/). Soils from three different fields that had a gradation in texture arising predominantly from erosional redistribution of clay down slope (Dungait et al. 2013) and different antecedent cropping were used in this work. They were soil with sandy clay (SC) texture that had most recently been under arable cropping (barley), and a sandy silt loam (SZL) textured soil also under an arable crop (barley). For these soils, the samples were taken from the 0-10 cm depth within the surface ploughed (Ap) horizon. The third soil had clay loam (CL) texture and had been under perennially cut and reseeded grassland and the sample was taken from the 0-10 cm depth in the A horizon.

171 **Experimental set-up**

172 Biochar (Bodfari Environmental, St. Asaph, UK) was produced from the pyrolysis of
173 hardwoods (400 °C, 24 h), primarily beech (*Fagus* spp.), and to a lesser extent ash (*Fraxinus*
174 *excelsior*), oak (*Quercus* spp.), birch (*Betula* spp.) and cherry (*Prunus* spp.). Pyrolysis was
175 conducted in a ring kiln by heating feedstock initially to 180 °C to allow release of volatile
176 gases, and subsequently to 400 °C for 24 hours. Soil and biochar characteristics, the latter
177 determined by Case et al. (2012), are summarised in Table 1. This wood-derived biochar was
178 chosen because it was produced using a feedstock and method that could realistically be applied
179 within a temperate agricultural context and because of its use in previous studies (Beesley et al.
180 2010; Case et al. 2012).

181 Mesocosms were constructed in plastic pots (volume = 38 L, 38 x 38 x 30 cm) with the bottom
182 10 cm filled with slate chippings to aid water drainage (Fig.1S). Soils were mixed and placed
183 into these pots from 5–9 May 2011. Biochar was sieved to remove particles >2 cm in size, and
184 mixed with half of each soil type equivalent to 2.0 % of soil dry weight, using spading forks for
185 a standardised duration. Soil that did not contain biochar was mixed in the same manner to
186 ensure consistent levels of physical disturbance across treatments. Each mesocosm received
187 the wet-weight equivalent of 25.2 kg dry soil, thus 2 % biochar-treated mesocosms contained
188 25.7 kg total substrate. Soil or soil-biochar mix was added to pots in four equal portions and
189 lightly compacted by hand between each addition to ensure even compaction throughout the
190 profile.

191 Crop seeds were sown on 11 May 2011. Optic barley (*Hordeum vulgare* L.) was planted at a
192 seeding rate of 1.8 t ha^{-1}, equivalent to half the typical UK rate (Dupuy et al. 2010), to allow
193 for the relative shallowness of the soil. Seeds were sown 1 cm deep in three rows spaced 12
194 cm apart. Perennial ryegrass (*Lolium perenne*) was sown at a rate of 2.0 t ha^{-1} by distributing

seeds evenly across the soil surface. The high seed density was chosen to account for seed loss due to wind and run-off in water. Neither seed type was fungicide-treated to avoid altering the soil food web. Mesocosms assigned to the unvegetated (control) treatment were weeded intensively by hand twice per month to prevent weed colonisation and maintain this control, at the same time weeds were also removed from the barley & ryegrass treatments. Optic barley and perennial ryegrass were re-seeded in May of 2012 and 2013.

The mesocosms were unfertilised because we decided that fertilisation would complicate an already complex experimental design and addition of artificial fertiliser (NPK) would only be field-realistic for the barley treatment. Mesocosms were placed in an outdoor enclosure (Fig.1S) to keep out herbivores (rabbits, deer) and so experienced ambient photoperiod and rainfall conditions (Scottish Environment Protection Agency weather station: Bush Estate weather station 55.86190844, -3.206554074; Annual mean precipitation ± SD: 2011 = 82.28 ± 31.43; 2012 = 93.17 ± 49.33; 2013 = 57.87 ± 42.88). During a period of relatively low precipitation from 13 May to 23 August 2013 (Mean ± SD: 2011 = 97.95 ± 39.54; 2012 = 130.4 ± 48.15; 2013 = 45.15 ± 27.27), we carried out once-weekly supplemental *ad libitum* watering of each mesocosm for a standard time period (10 s^{-1} mesocosm).

Soil pH and chemical composition

To measure the impact of biochar on soil chemistry (Table 2), soil was sampled in August 2011, 2012 and 2013. A single soil sample was taken from each mesocosm (3.5 cm Ø core to 10 cm depth), dried (105°C ± 5, 24 h) and homogenised in a ball mill, then sieved (2 mm mesh). Soil moisture was calculated by weighing the soil prior to and after the oven drying process. Soil pH was determined by combining a 1g subsample of dried, milled soil with 2 ml deionised water. This suspension was placed on a rotary shaker for 30 minutes, then allowed to settle for 30 minutes. Finally, the mixture was manually shaken for 30 seconds prior to analysis using a

pH probe (Mettler-Toledo, Columbus, USA). Subsamples (30 mg) of dried, sieved soil were analysed for total carbon and nitrogen content (%) using flash combustion at 950 °C in an elemental analyser (EL Cube, Elementar, Hanau, Germany).

To understand the impact of biochar addition on soil carbon balance (i.e. whether it is stabilised or primed for release by promotion of microbial activity) the values of total carbon content obtained from each biochar-treated soil were adjusted by subtracting the amount of carbon introduced to the soil as biochar, using Equation 1:

Equation 1. $C_A = (C_T - 0.02*C_B)/0.98$

C_A represents adjusted carbon content, which is the percentage carbon content of the soil after subtracting for the theoretical amount of biochar carbon added to the soil. C_T represents total measured percent carbon in the biochar-treated soil sample (i.e. the observed percentage carbon content of the biochar-soil mix). C_B represents the percentage carbon content of pure biochar (72.3%), which was multiplied by the dose rate of 0.02 (2% w/w of total substrate). The aim of this analysis was to determine whether carbon had been lost from the biochar-treated substrate – if this were the case, C_A for biochar-treated soils would be less than the percentage carbon content of the corresponding control soils. This would signify loss of either biochar carbon (via mineralisation of the labile portion) or soil carbon (via biochar-induced priming).

Soil microbial community structure

Phospholipid fatty acid (PLFA) analysis was used in order to quantify the dry weight-based mass of markers for microbial biomass and fungal-to-bacterial ratio in the soil in different treatments (Frostegård et al. 2011). One soil sample per mesocosm (3.5 cm Ø core to 10 cm depth) was taken in August 2013 and stored at -20 °C prior to freeze-drying at -20 °C. A subsample (1g) of the freeze-dried soil was subsequently taken for phospholipid fatty acid (PLFA) analysis. Three measures of microbial community structure were derived. Total PLFA

provided a measure of overall microbial biomass; the 16:1ω5 fatty acid marker was used as a proxy measurement for arbuscular mycorrhizal biomass (Ngosong et al. 2012; Olsson et al. 1995); and the fungal-to-bacterial PLFA ratio was calculated by dividing the fungal PLFA marker (18:2ω6,9) by the summed bacterial PLFA markers (i15:0, a15:0, 15:0, i16:0, 16:1ω7, a17:0, i17:0, cy17:0, cis18:1ω7, cy19:0).

Soil invertebrate abundance

To assess the impact of biochar on the soil invertebrate abundance, soil was sampled for nematodes on 21-22 June 2011, 28-29 August 2012 and 20 August 2013, and for microarthropods (collembola, mites) on 20 August 2013. On each occasion, each mesocosm was sampled in three random locations with a 3.5 cm Ø corer to 10 cm depth. The empty space created by soil coring was filled with a cylindrical pipe of the same diameter, to avoid altering the soil bulk density or coring in a location that had been previously sampled. Each soil core was split vertically into two halves, one half designated for nematode extraction and the other for microarthropod extraction. The three replicate halves were pooled into a single sample for each pot, with fresh weight recorded prior to invertebrate extraction. For nematode extraction, soil samples were placed in a Baermann funnel system for 24 hours wet extraction. Microarthropods were collected into alcohol-filled vials using Tullgren funnels (Burkard Scientific, Uxbridge, UK) for 24 hours. Following extraction of invertebrates, the soil was oven-dried (105 ± 5 °C, 24h) and weighed to determine soil dry weight. Nematodes and microarthropods (mites and collembola) were counted under a light microscope, and abundance values were converted to standardised densities by calculating individuals per g of dry soil.

Crop plant production

To quantify annual aboveground primary production, barley and ryegrass biomass was collected by cutting the vegetation biomass to 1 cm above the soil surface using handheld shears

267 in September of each year, 2011-2013. Root biomass was determined in August 2013 by taking
268 one soil core from each mesocosm (3.5 cm Ø, 10 cm depth). Only the top 10 cm were analysed
269 so that root data would correspond to the same soil stratum as sampled for invertebrates.
270 Separation of roots from soil was accomplished using washing, sieving (1mm mesh) and
271 handpicking. Once separated, the plant material was oven-dried (70 °C, 24 h) prior to weighing.

272 **Ecosystem carbon dioxide fluxes**

273 Ecosystem respiration and net ecosystem exchange (NEE) of CO_2 fluxes from each mesocosm
274 were quantified monthly (Orwin et al. 2014). An IRGA EGM-4 (PP Systems, Herts, UK)
275 connected to a gas sampling chamber (45,693 cm^3) was used. The chamber was inlaid with
276 Propafilm C on all five sides to allow light transmission so NEE of CO_2 could be measured.
277 Ecosystem respiration was measured by using an aluminium cover to exclude light from the
278 chamber. Prior to the onset of the experiment, chamber airtightness was confirmed by injecting
279 a known concentration of SF_6 into a chamber connected to a trial pot, then using a gas
280 chromatograph (Hewlett Packard 5890 Series II, Palo Alto, USA) to monitor SF_6 levels over
281 the course of one hour.

282 The volume of substrate (soil or soil/biochar mix) within mesocosms varied slightly based on
283 soil type and presence or absence of biochar. To account for differences in headspace between
284 the soil surface and the top of the pot, this volume was measured in each pot, and added to the
285 chamber volume value at the time of sampling. Net CO_2 efflux data were expressed as positive
286 values whereas net CO_2 uptake data were expressed as negative values.

287 **Statistical analysis**

288 Prior to analysis of the biological effects of experimental biochar addition, we used paired t-
289 tests to determine whether edaphic properties (soil carbon, nitrogen and moisture content, soil

carbon to nitrogen ratio, and soil pH) were significantly modified by biochar treatment across crop and soil treatments (Table 2).

Biotic and ecosystem responses to experimental treatments and covariates were analysed with linear mixed models (LMMs) (proc MIXED, SAS Institute, Cary, USA). Ecosystem response variables modelled were: NEE, ecosystem respiration, plant biomass (aboveground and root), and soil carbon content. Soil biological response variables were: fungal-to-bacterial ratio, total PLFA, and densities of nematodes, collembolans, and mites, respectively. Explanatory variables in each model always included the three experimental treatments: biochar (+/-), plant type (barley, ryegrass, unvegetated) and soil texture (sandy clay, sandy silt loam, clay loam). Covariates in the candidate list of explanatory variables included: fungal to bacterial ratio, soil pH, soil nitrogen content (%), soil moisture content (%), and densities of nematodes, mites, and collembola. Fitting of these covariates was contingent on being appropriate for the particular LMM in question i.e. a meaningful ecological predictor. We detail these exceptions below.

For response variables with annual repeated measures (aboveground plant biomass, nematode density, NEE, ecosystem respiration, soil carbon content) the sampling year was included as an additional categorical fixed effect. Models of above- and belowground plant biomass responses excluded replicates from the unvegetated treatment (no plant growth due to intensive manual weeding). Soil carbon content and soil carbon to nitrogen ratio were not included in the candidate list of explanatory variables because of a strong correlation (Spearman's rank $p < 0.0001$) with soil nitrogen content (Table 1S). Soil pH was not included as an explanatory variable in soil carbon models as it was considered a result, rather than a cause, of soil chemical composition (Table 1S). Soil fungal to bacterial ratio and total PLFA were very strongly correlated (Table 1S), hence only fungal to bacterial ratio was fitted as a covariate in mixed models. To account for seasonality in models of NEE and ecosystem respiration, Julian date was also included as a covariate. The date was transformed using the functions

315 $\sin(2\pi * d/365.25)$ and $\cos(2\pi * d/365.25)$, where *d* represented a Julian date between 1 and 366.
316 Sine and cosine of Julian date were always fitted together into a model, and retained if either or
317 both were significant.

318 Pairwise interactions between the three treatments, and between treatments and covariates, were
319 fitted to the LMMs, but interactions between pairs of covariates were not tested. All LMMs
320 included spatial block as a random effect and an autoregressive AR(1) structure at the
321 mesocosm level to account for any repeated measures. Aboveground plant biomass and
322 nematode density were log-transformed to meet LMM assumptions of homogeneity of variance
323 and normally distributed residuals. Satterthwaite's approximation was used to estimate degrees
324 of freedom. Final model selection was achieved by forward stepwise and backward elimination
325 of least significant terms; where these two methods did not converge (aboveground plant
326 biomass, nematode density) the forward stepwise-selected model was presented as the most
327 conservative option. Non-significant main effects were only retained in the final model where
328 they were part of a significant pairwise interaction. We report type III (adjusted) F and p values
329 of all treatments, covariates and two-way interactions when significant ($\alpha = 0.05$). Full tables of
330 LMM giving all results of tests including >0.05 are reported in supplementary materials (Tables
331 2S-6S). Bonferroni-adjusted LS means comparisons are presented graphically to show the
332 effects of experimental treatments. Partial residual plots were used to illustrate the effects of
333 significant covariates conditional on the random effects and other significant explanatory
334 variables (and the interactions thereof) within the final model.

335 **Results**

336 **Edaphic properties**

Biochar addition significantly altered the edaphic properties of the soil across all soil and crop treatments by increasing the pH, moisture content, % carbon content, and soil carbon-to-nitrogen (CN) ratio of the soil, but not the % nitrogen content (Table 2).

Soil carbon (C) content

Overall soil carbon content was increased by biochar treatment (Fig. 1, Tables 1 & 3) in accord with its high carbon content (Table 1). However, the impact of biochar-induced changes to soil carbon differed between soil textures, with biochar-associated increase in soil carbon greatest in CL soil (mean ± S.E = 1.32% of soil mass ± 0.12), compared to SC (0.84% ± 0.10) and SZL (0.66% ± 0.09) soil (Table 3). Biochar did not interact directly with any other experimental treatment or covariate to affect soil carbon content (see Table 2S for all tests).

Soil texture and crop plant type also affected soil carbon content (Fig. 1, Table 3). Soil carbon was highest in the sandy silt loam (SZL) and lowest in sandy clay (SC) soil (Table 3) and under perennial ryegrass (mean ± S.E. = 3.52 % ± 0.12) compared to barley (3.32 % ± 0.12) and unvegetated (3.30 % ± 0.11) treatments (Table 3). There was no evidence of biochar-induced changes to soil biodiversity affecting soil carbon content (Table 2S). However, nematode density was inversely related to soil carbon (Table 3), but this also varied with soil texture with the greatest effect observed in CL soil compared to other soil textures (Table 3 – Nematoda × soil texture).

Non-biochar derived soil carbon (C_A) content.

Controlling for the mass of carbon introduced to each mesocosm in the form of biochar itself (Equation 1) revealed a strong influence on the amount of non-biochar derived soil carbon (C_A) of biochar, soil type and their interaction (Fig. 1 & 2, Table 3). Biochar addition was associated with an overall loss of carbon (C_A) from the top 10 cm of soil (from which samples were taken) compared to control, with some variation between soil textures (Fig. 2). Again there was no

361 evidence of biochar-induced changes to biodiversity influencing non-biochar derived soil
362 carbon (Table 2S: C_A), but collembolan density related positively and highly significantly with
363 non-biochar derived soil carbon content (Table 3: C_A).

364 **Microbial community structure**

365 Soil fungal to bacterial ratio increased significantly in the presence of biochar (Fig. 1 & 3a,
366 Table 4), but total PLFA was unaffected (Fig. 3a, Table 3S). Biochar treatment as a main effect
367 had no influence on the mean mass of the 16:1ω5 fatty acid marker of AM fungal biomass (Fig.
368 1, Table 3S). There was, however, a positive relationship between the mass of the 16:1ω5 fatty
369 acid marker and collembolan density in the biochar-treated mesocosms (Fig. 4b, Table 3S:
370 $F_{(1,64)} = 6.35$, $p = 0.014$). Biochar did not interact directly with any other experimental treatment
371 or covariate to affect the microbial community (see Table 3S).

372 Crop plant treatment had a strong influence on the microbial community (Fig. 1, Table 3S).
373 Total PLFA and the 16:1ω5 fatty acid marker of AM fungal biomass were both significantly
374 greater under ryegrass than barley, both of which were higher than the unvegetated control (Fig.
375 3b, Fig. 4a, Table 3S). While the fungal to bacterial ratio was elevated under ryegrass (Fig.
376 3b), it was not statistically significant after accounting for other model parameters (Table 4).
377 Total PLFA and fungal to bacterial ratio were significantly affected by the interaction between
378 crop type and mite density (Acari) reflecting greater densities under ryegrass (PFLA - Table
379 3S: $F_{(2,60)} = 8.44$, $p = 0.0006$; Fungi:Bacteria - Table 4).

380 Total PLFA and fungal to bacterial ratio (Spearman correlation coefficient = 0.70, Table 1S)
381 differed significantly among soil textures with the lowest ratio found in the sandy clay (SC)
382 (Fig. 3c, Table 4, Table 3S). The impact of soil texture on the 16:1ω5 fatty acid marker of AMF
383 mass was also highly significant (Table 3S), with the highest level in soil SC (1409 ± 98 ng g^{-1}
384 dry soil, mean ± S.E.) compared to soil CL (1370 ± 98 ng g^{-1} dry soil) and soil SZL (1213 ± 99

385 ng g^{-1} dry soil). A significant interaction between soil texture and soil moisture also influenced
386 the fungal to bacterial ratio, mainly due to a negative relationship in soils SZL and CL (Table
387 4).

388 Soil fungal to bacterial ratio was negatively related with soil pH (Table 4) indicating a greater
389 relative abundance of fungi in the more acidic conditions (Table 1). Soil nitrogen content (%)
390 had a marginally significant negative impact on soil fungal to bacterial ratio (Table 4).

391 **Soil invertebrate abundance**

392 Biochar addition over the three years of the experiment had no direct effect on the densities of
393 soil invertebrates. Although biochar appeared to reduce soil nematode density, this was not
394 statistically significant at $\alpha = 0.05$ (Fig. 3a, Table 4S: $F_{(1,97)} = 4.00$, $p = 0.048$). The density of
395 collembolans or mites was unaffected by biochar treatment of the soil (Fig. 1 & 3a, Table 4S).

396 In contrast, the crop and soil texture treatments profoundly affected densities of nematodes and
397 collembolans, although mite densities were unaffected (Fig. 1 & 3b-c, Table 4S). Mesocosms
398 sown with ryegrass supported a higher nematode ($F_{(2,91)} = 11.78$, $p < 0.0001$) and collembolan
399 ($F_{(2,91)} = 9.64$, $p = 0.0002$) density than either barley-planted or unvegetated mesocosms (Fig.
400 3b, Table 4S). Nematode density was greatest in the sandy clay (SC) and lowest in the clay
401 loam (CL) soils ($F_{(2,150)} = 11.38$, $p < 0.0001$); whereas collembolan density was significantly
402 greater ($F_{(2,64)} = 3.94$, $p < 0.024$) in the SZL soil (Fig. 3c, Table 4S).

403 Overall soil texture also affected nematode and collembolan densities in interaction with crop
404 plant type and soil covariates. Nematode density related positively to soil moisture ($F_{(1,187)}$
405 $=7.28$, $p < 0.008$), but the slope of this relationship increased from CL to SZL to SC soils,
406 respectively (Table 4S). Nematode density was also affected by the interaction between soil
407 texture and crop plant type (Table 4S), with the greatest nematode density in ryegrass-planted
408 SZL mesocosms (mean ± S.E 5.02 individuals g^{-1} soil ± 0.81) and the lowest in unvegetated

409 CL mesocosms (0.67 individuals g^{-1} soil ± 0.12). Collembolan densities were affected by the
410 interaction between soil texture and pH ($F_{(2,64)}$ =3.97, p< 0.024), with a positive relationship
411 between densities and pH in CL soil and a negative relationship in SZL & SC soils (Table 4S).

412 **Crop plant production**

413 We detected no effect of biochar on crop biomass either aboveground or on roots (Fig. 1, Table
414 S5), either as a main effect or interaction. Aboveground biomass yield was significantly greater
415 in the barley (mean ± S.E. = 36.0 g^{-1} y^{-1} ± 5.4) than the perennial ryegrass (8.08 g^{-1} y^{-1} ± 0.8)
416 treatment; but root biomass did not differ between the crop treatments (Table 5S). Soil texture
417 had a strong effect on aboveground primary production (Table 5S: $F_{(2,101)}$ = 6.68, p = 0.002),
418 with the highest aboveground plant biomass per mesocosm in soil SC (38.7 g^{-1} y^{-1} ± 8.1) and
419 significantly lower production in soils SZL (13.9 g^{-1} y^{-1} ± 2.1) and CL (13.8 g^{-1} y^{-1} ± 2.0). Root
420 biomass was, however, not directly affected by soil texture (Table 5S).

421 Aboveground plant biomass was related negatively to nematode density, while root biomass
422 increased with nematode density under barley (Table 5S). Overall, aboveground plant biomass
423 related positively to soil mite density (Table 5S: $F_{(1,58)}$ = 4.52, p = 0.038), driven by an
424 interaction with crop type with barley supporting greater densities (Table 5S: $F_{(1,58)}$ = 4.27, p =
425 0.043). Crop production was further complicated by negative interactions between mite
426 densities and soil texture both aboveground (Table 5S: $F_{(2,57)}$ = 3.68 p = 0.031) and for roots
427 (Table 5S: $F_{(2,30)}$ = 12.21, p = 0.0001).

428 **Ecosystem carbon dioxide fluxes**

429 Biochar treatment had no significant effect on ecosystem respiration or NEE, whereas both crop
430 plant species and soil texture had a large influence on these parameters (Fig. 1, Table 6S). NEE
431 was significantly affected by crop species (Table 6S: $F_{(2,299)}$ = 6.07, p = 0.003). The greatest
432 CO_2 uptake (indicated by a negative g CO_2 m^{-2} h^{-1}) was seen in ryegrass mesocosms (mean ±

S.E. = -0.33 g CO_2 m^{-2} h^{-1} ± 0.02) compared to barley (-0.25 g CO_2 m^{-2} h^{-1} ± 0.02) and unvegetated (-0.12 g CO_2 m^{-2} h^{-1} ± 0.01) mesocosms. Ecosystem respiration was also affected by crop type (Table 6S: $F_{(2,185)}$ = 17.87, p<0.0001), with greater respiration rate in ryegrass mesocosms (-0.02 g CO_2 m^{-2} h^{-1} ± 0.005) than barley (-0.005 g CO_2 m^{-2} h^{-1} ± 0.005) and unvegetated (-0.0001 g CO_2 m^{-2} h^{-1} ± 0.005) mesocosms. Soil texture also affected NEE (Table 6S: $F_{(2,256)}$ = 7.48, p = 0.001), but not ecosystem respiration, with greater CO_2 uptake in mesocosms comprising SC soils (-0.22 g CO_2 m^{-2} h^{-1} ± 0.02) than SZL (-0.16 g CO_2 m^{-2} h^{-1} ± 0.01) and CL (-0.14 g CO_2 m^{-2} h^{-1} ± 0.01) soils.

Plant biomass was positively related with NEE (Table 6S: $F_{(1,316)}$ = 7.92, p = 0.001) and ecosystem respiration (Table 6S: $F_{(1,208)}$ = 25.09, p<0.0001), although for the latter an interaction with crop species revealed a negative relationship in the barley treatment (Table 6S: $F_{(1,207)}$ = 22.49, p<0.0001).

NEE was also influenced by an interaction between crop plant and soil pH with positive and negative relationships with pH under barley and ryegrass, respectively (Table 6S: $F_{(2,290)}$ = 3.27 p = 0.039). NEE was affected by the interaction of soil N content × crop type with a more positive slope in barley than ryegrass or unvegetated treatments (Table 6S: $F_{(2,338)}$ = 21.05, p<0.0001). Nematode density was a significant positive predictor of both NEE (Table 6S: $F_{(1,145)}$ = 8.08, p =0.005) and ecosystem respiration (Table 6S: $F_{(1,200)}$ = 6.38, p = 0.0123), but fungal: bacterial ratio and collembolan or mite densities were not related to ecosystem CO_2 fluxes (Table 6S).

Discussion

This study is among the first to assess experimentally and simultaneously the impact of biochar on multiple dimensions of soil biodiversity and ecosystem function in different temperate agricultural soils. Contrary to our predictions, and despite biochar-associated changes to

457 edaphic properties (Table 2), biochar addition did not cause any direct changes to soil
458 invertebrate abundance, carbon cycling or crop production over the three years of this
459 experiment. This suggests a high level of functional resistance of these particular soils to this
460 perturbation, at least for this type of biochar and in the spatio-temporal context of this
461 experiment.

462 Across all the tested soil textures, biochar treatment did increase water holding capacity and
463 soil pH (Table 2) along with elevating the relative abundance of fungi (c.f. our prediction (i)
464 that PLFA fungal-bacterial ratio would be lower). However, the lack of a statistical interaction
465 with biochar (Table 4) meant we were unable to explicitly link this shift in microbial community
466 dominance with biochar-driven changes in soil physico-chemical properties (Lehmann et al.
467 2011; McCormack et al. 2013). This observed increase in fungal dominance is consistent with
468 some biochar trials (see citations in Warnock et al. 2007), but contrasts with a UK field trial
469 that showed hardwood biochar reduced the soil fungal to bacterial PLFA ratio (Jones et al.
470 2012).

471 We found no evidence that biochar directly enhanced mycorrhizal fungal growth, indicated by
472 the 16:1ω5 fatty acid marker of AM fungal biomass in PFLA analysis (Table 3S), something
473 considered a likely consequence of the greater pore space provided by biochar or its
474 neutralization of acidic soil conditions (McCormack et al. 2013; Prendergast-Miller et al. 2014;
475 Warnock et al. 2007). However, the level of this marker of AM fungal biomass related
476 positively to collembolan abundance in the presence of biochar, but not in the controls. We
477 speculate that this might indicate biochar modulation of collembolan grazing of AM fungi: the
478 complex architecture of biochar surfaces may have provided physical refuges from fungal
479 grazers or led to intermediate grazing pressure that can stimulate compensatory AM fungal
480 growth (Bretherton et al. 2006; McCormack et al. 2013; Warnock et al. 2007). AM fungi have
481 an important role in soil carbon sequestration (Zhu and Miller 2003). While a large proportion

of hyphal biomass is rapidly turned over leading to carbon loss via mineralization, more stable fungal components (e.g. chitin, glomalin) have a longer residence time (Staddon et al. 2003; Zhu and Miller 2003). Although it remains to be proven, if biochar-modulation of fungivory can lead to sustained increases in AM fungal biomass then this could represent a mechanism of biochar-induced soil carbon stabilization and sequestration. Therefore, one interpretation is that subtle changes to biotic interactions by biochar treatment may explain the shift in fungal dominance and interaction with collembolan density observed in this experiment. However, as with vegetation, the response of microbial community composition to biochar is likely to be context-dependent and temporally dynamic (Hol et al. 2017).

Soil invertebrate abundance was generally unaffected by biochar treatment (Fig. 1 & 3a). Although there was an apparent reduction in nematode density in this experiment, it was not statistically significant and of small magnitude compared to crop plant and soil effects. The lack of impact on nematode densities corresponded to the general lack of biochar-induced effects on root biomass or total microbial PLFA, both food resources for plant parasitic or microbial feeding nematode taxa (Yeates et al. 1993). Our findings thus support the lack of an impact of hardwood biochar on nematode survival seen in a short-term microcosm study (Hagner et al. 2016) and on nematode biomass in a one-year trial in a maize agroecosystem (Pressler et al. 2017). However, reductions in the abundance of a plant parasitic nematode species (George et al. 2016) have been reported elsewhere, as have alterations to nematode abundance and community composition associated with toxic polycyclic aromatic hydrocarbons and heavy metals contained in some biochar products (Chen et al. 2009). This discrepancy among studies is thus likely to be due to the specific ecological contexts, biochar feedstock, product variability and contaminants, and experimental design (e.g. duration). Microarthropods (Acari, Collembola) were also unaffected by both biochar treatment and, contrary to our prediction (ii), the observed biochar-associated shift in fungal to bacterial ratio. Although few other studies

507 have thus far addressed the effect of biochar on soil microarthropods, increased collembolan
508 reproduction has been reported in laboratory bioassays involving biochar made from hardwood
509 (Marks et al. 2014) and maize crop residues (Hale et al. 2013).

510 The intrinsic carbon content of biochar meant that once applied it leads to increased soil carbon
511 content. However, estimating the 'adjusted' carbon content (C_A) to account for the amount of
512 carbon added to the system as biochar revealed that biochar-treated soils contained significantly
513 less carbon than the control soils. This might be a consequence of biochar loss from soils due
514 to leaching or wind transport, as has been reported elsewhere (Major et al. 2010). This
515 possibility is supported by a significant interaction in the adjusted carbon model (Table 3)
516 indicating the different level of 'adjusted' biochar carbon content relative to the control for each
517 soil texture. The implication being that the properties of the different soil textures (e.g. surface
518 roughness, aggregate or pore size) may have influenced the magnitude of leaching/erosion
519 losses. However, because we did not quantify soil carbon content of samples taken from deeper
520 in the soil profile (i.e. >10cm depth) we are unable to determine if vertical transmission of soil
521 carbon through the soil profile occurred. Although we cannot exclude this possibility, it should
522 be noted we did not detect an effect of experimental year in our models, which implies a lack
523 of change in soil carbon content sampled from the upper layer (<10cm depth) over time.

524 Alternatively, carbon may have been lost from biochar-treated mesocosms *via* either
525 mineralisation of biochar carbon or biochar-induced priming of soil carbon (Bruun et al. 2014;
526 Liu et al. 2016; Maestrini et al. 2015). If biochar-induced soil carbon priming occurred, this
527 may have happened during the initial weeks between biochar treatment and the first soil carbon
528 sampling, rather than during the experiment because there was no statistical effect of 'year' or
529 its interaction with biochar in our soil carbon content models. Alternatively, for mineralisation
530 or priming to explain the loss of carbon from biochar-treated mesocosms it could have occurred

on a finer timescale than could be detected by our monthly CO_2 flux measurements as we found no significant impact of biochar on ecosystem respiration rate.

Although there was no interactive effect between biochar and soil biota on soil respiration (prediction iii), nematode density did have a significant positive effect on ecosystem respiration. Direct mineralisation of carbon by nematode activity is unlikely or at a low level, more probably this peak may have resulted from stimulation of microbial activity by enhanced nematode grazing and/or decomposition of plant and microbial biomass (Gebremikael et al. 2016; Yeates et al. 1993). However, we did not detect any other relationships in the measured components of soil biodiversity so the underpinning mechanism remains unclear.

Biochar had no effect on shoot and root production of barley and perennial ryegrass, which may imply there is little agricultural yield penalty if biochar is added to these soils (Bargmann et al. 2013). Biochar also had no effect on the complex relationships we detected between soil fauna (abundance of nematodes and mites) and plant biomass production in different soil textures or crop species. The overall lack of a biochar effect on plant production and NEE indicates that carbon cycling within the system tested here was generally robust to the addition of biochar within the time span of the study. Moreover, our results imply that biochar had little or no impact on the biodiversity-function relationships in this study system. Trophic interactions of soil invertebrates can modulate soil decomposition processes and it is possible the high functional redundancy of soil biological communities buffered soil carbon fluxes against the effects of biochar in this study (Ayres et al. 2010; Bardgett and van der Putten 2014; Bruun et al. 2014; Heemsbergen et al. 2004; Jenkins et al. 2017; Rousk et al. 2009)

Altogether, this points to the apparent robustness of these systems to biochar perturbation, but also the importance of understanding the performance and food web dynamics of these systems in different agri-environmental contexts or under other stresses (Backer et al. 2016; Bardgett

555 and van der Putten 2014; McCormack et al. 2013; McKenzie et al. 2016). There are,
556 nonetheless, some caveats to our experiment. Although run over three years, it remains a short-
557 term snapshot of experimental mesocosms. Furthermore, other field-realistic aspects were not
558 included in the experimental design, for instance there was no use of chemical fertilizers or
559 simulation of tillage or crop rotation. In examining biotic responses we only used one method
560 (PFLA) for assessing soil microbial changes with known limitations (Frostegård et al. 2011;
561 Ngosong et al. 2012; Olsson et al. 1995) and which does not identify more subtle phylogenetic
562 or functional shifts in the microbial community. Similarly, we only measured overall taxon
563 abundance and not responses of invertebrate functional diversity or different trophic groups to
564 biochar, which may have revealed other effects. Consequently, due caution is needed when
565 translating these experimental results to real agroecosystems without suitable additional trials.

566 The lack of biochar effects on soil biodiversity and ecosystem functions over the course of this
567 multi-year study may provide further evidence for the claims made that biochar in soil is largely
568 inert (Lehmann 2007; Lehmann et al. 2011). The ability of biochar to cause minimal disruption
569 to soil biodiversity and processes, while acting as a stable stock of soil carbon, may be the most
570 important determinant of its successful implementation (Smith 2016). There remains a clear
571 need, however, to understand better the effects of biochar soil amendment on different
572 components of soil biodiversity, including above-belowground biotic interactions, in order to
573 gauge the potential for more subtle effects on biotic controls of ecosystem production and CO_2
574 fluxes.

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Data statement. Raw data will be archived at the NERC Environmental Information Data Centre http://eidc.ceh.ac.uk/. Summary data (means + SE) for soil invertebrate densities, above-belowground crop biomass and PFLA are contained in online resources linked to this article (Tables 7S-9S).

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Table 1. Initial physical and chemical properties of the agricultural soils and biochar used in this experiment. For further analytical results of the biochar used see Case *et al.* (2012). The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil - the role of soil aeration. *Soil Biology and Biochemistry,* 51**,** 125-134.

	Soil SC	**Soil SZL**	**Soil CL**	**Biochar**
Texture	Sandy clay	Sandy silt loam	Clay loam	n/a
Total C (%)	1.93 ± 0.04 (n = 12)	3.85 ± .16 (n = 12)	2.67 ± 0.08 (n = 12)	72.3 ± 0.15 (n = 3)
Total N (%)	0.14 ± 0.01 (n = 12)	0.16 ± 0.01 (n = 12)	0.12 ± 0.01 (n = 12)	0.71 ± 0.001 (n = 3)
CN ratio	13.8 ± 0.7 (n = 12)	24.1± 0.1 (n = 12)	22.3 ± 0.8 (n = 12)	102
pH	5.56 ± 0.04 (n = 12)	6.40 ± 0.04 (n = 12)	6.11 ± 0.01 (n = 12)	9.25 ± 0.04 (n = 4)

Table 2. Effects of biochar on soil chemical properties across soil and plant treatments in the experiment. Values are means ± standard error (S.E.) and t and *p*-values are the result of a paired t-test using data collected from all mesocosms (n = 72) in each year of the experiment (n = 3).

	Biochar	Control	t	*p*
Soil pH	6.45 ± 0.03	6.15 ± 0.03	6.63	< 0.0001
Soil moisture (%)	18.43 ± 0.36	17.20 ± 0.34	2.51	0.0128
Soil carbon (%)	3.28 ± 0.09	2.92 ± 0.08	8.04	< 0.0001
Soil nitrogen (%)	0.20 ± 0.01	0.20 ± 0.01	0.11	0.9096
Soil CN ratio	20.81 ± 0.78	15.64 ± 0.55	5.40	< 0.0001

Table 3. Final linear mixed model of the response of soil carbon content (total C and adjusted C_A to account for the proportion of C added to the soil as biochar) to experimental treatments and covariates. Values are estimates of fixed effects and type III (adjusted for other significant terms) *F* & *p* statistics $\alpha = 0.05$. Annual measurements of soil carbon (n = 3) at the mesocosm level were accounted for using an autoregressive AR(1) structure. × = interaction. Biochar (+) vs Control (−); SZL: sandy silt loam, CL: clay loam, SC: sandy clay; Collembola or Nematoda = density of these soil invertebrates.

Response	Fixed effect	Class	Estimate	$F_{(ndf, ddf)}$	*p*
Soil carbon (C) content	Intercept		2.385 ± 0.236		
Random effects:	Soil texture	SZL	1.59 ± 0.17	$63.04_{(2,125)}$	<0.0001
Spatial block = 0.0005		CL	0.78 ± 0.18		
Mesocosm AR(1) = 0.008		SC	0		
Residual variance = 0.278	Biochar	+	0.82 ± 0.13	$155.33_{(1,71)}$	<0.0001
		−	0		
	Nematoda		-0.0004 ± 0.021	$6.80_{(1,182)}$	0.010
	Crop type	Barley	-0.59 ± 0.28	$3.94_{(2,69)}$	0.024
		Ryegrass	0.13 ± 0.28		
		Unvegetated	0		
	Biochar × soil texture	+ × SZL	-0.14 ± 0.19	$7.28_{(2,72)}$	0.001
		+ × CL	0.55 ± 0.19		
		+ × SC	0		
		− × SZL	0		
		− × CL	0		
		− × SC	0		
	Nematoda × soil texture	SZL	0.006 ± 0.032	$4.72_{(2,182)}$	0.010
		CL	-0.201 ± 0.070		
		SC	0		
Adjusted soil C_A content					
Random effects:	Soil texture	SZL	1.60 ± 0.13	$128.00_{(2,77)}$	<0.0001
Spatial block = 0		CL	0.58 ± 0.13		
Mesocosm AR(1) = 0.073		SC	0		
Residual variance = 0.287	Biochar	+	-0.71 ± 0.13	$63.62_{(1,77)}$	<0.0001
		−	0		
	Collembola		0.78 ± 0.29	$7.25_{(1,78)}$	0.009
	Biochar × soil texture	+ × SZL	-0.141 ± 0.189	$4.26_{(2,78)}$	0.018
		+ × CL	0.403 ± 0.190		
		+ × SC	0		
		− × SZL	0		
		− × CL	0		
		− × SC	0		

Table 4. Final linear mixed model of the response of soil fungal to bacterial ratio (PLFA analysis) to treatments, covariates and their interactions. Values are estimates of fixed effects and type III (adjusted for other significant terms) *F* & *p* statistics α =0.05. × = interaction. Biochar (+) vs Control (−); SZL: sandy silt loam, CL: clay loam, SC: sandy clay; Acari = mite density..

Response variable	Fixed effect	Level	Estimate	$F_{(ndf, ddf)}$	*p*
Fungal-to-bacterial ratio	Intercept		3.709 ± 1.126		
	Crop type	Barley	-0.752 ± 0.439	$3.00_{(2,54)}$	0.058
Random effects:		Ryegrass	-1.097 ± 0.466		
Spatial block = 0		Unvegetated	0		
Residual variance = 0.024	Soil texture	SZL	2.306 ± 0.588	$8.70_{(2,54)}$	0.0005
		CL	1.559 ± 0.583		
		SC	0		
	Biochar	+	0.196 ± 0.067	$8.53_{(1,54)}$	0.005
		–	0		
	Acari		0.016 ± 0.345	$3.01_{(1,54)}$	0.089
	Soil pH		-0.673 ± 0.204	$10.92_{(1,54)}$	0.002
	Soil N		-0.958 ± 1.950	$4.22_{(1,54)}$	0.045
	Soil moisture		0.045 ± 0.024	$1.68_{(1,54)}$	0.201
	Acari × crop type	Barley	-0.507 ± 0.413	$10.77_{(2,54)}$	0.0001
		Ryegrass	1.468 ± 0.467		
		Unvegetated	0		
	Soil N × crop type	Barley	4.977 ± 2.444	$3.77_{(2,54)}$	0.029
		Ryegrass	6.610 ± 2.538		
		Unvegetated	0		
	Soil moisture × soil texture	SZL	-0.131 ± 0.041	$5.59_{(2,54)}$	0.006
		CL	-0.083 ± 0.040		
		SC	0		

Fig. 1. Graphical summary of effect sizes of biochar treatment (+/-), crop plant species (barley, ryegrass, unvegetated) and soil texture (sandy clay, sandy silt loam, clay loam) on ecosystem parameters in a three-year mesocosm experiment (2011-2013). Shading indicates the F-ratio (scaled by ln transformation to aid visual clarity) from LMMs of the experimental treatments for each ecosystem parameter and statistical significance is denoted by * < 0.05, **< 0.001, ***<0.0001.

Fig. 2. The interaction between biochar and soil texture affecting adjusted (C_A) soil carbon content accounting for carbon introduced in the form of biochar (see equation 1 in method). Dark grey bars = biochar-treated mesocosms, white bars = control mesocosms. SC = sandy clay; SZL = sandy silt loam; CL = clay loam. Values are means of raw data (control) and adjusted raw data (biochar treatment) ± standard error.

Fig. 3. Response of soil biota to (a) biochar, (b) crop type and (c) soil type. Coll. = collembola; Nem. = nematodes, F:B = soil fungal-to-bacterial ratio. Nematodes, collembola and mites are expressed as organism density (individuals g^{-1} dry soil). Total PLFA is expressed as ng PLFA g^{-1} dry soil. All show the results of a Bonferroni-adjusted LS means comparison (± standard error, S.E.) produced from an LMM using block as a random effect. In the case of nematode density repeated annual measures (n = 3) at the mesocosm level were accounted for using an AR(1) structure.

Fig. 4. Response of fatty acid marker of arbuscular mycorrhizal mass (16:1ω5 ng g^{-1} dry mass of soil) to a) crop type and b) the interaction between collembolan density and biochar. Data presented are a) LS means ± S.E. and b) partial residuals plotted on the linear predictor scale. Filled symbols and solid line, biochar; open symbols and dashed line, control.

Fig. 1

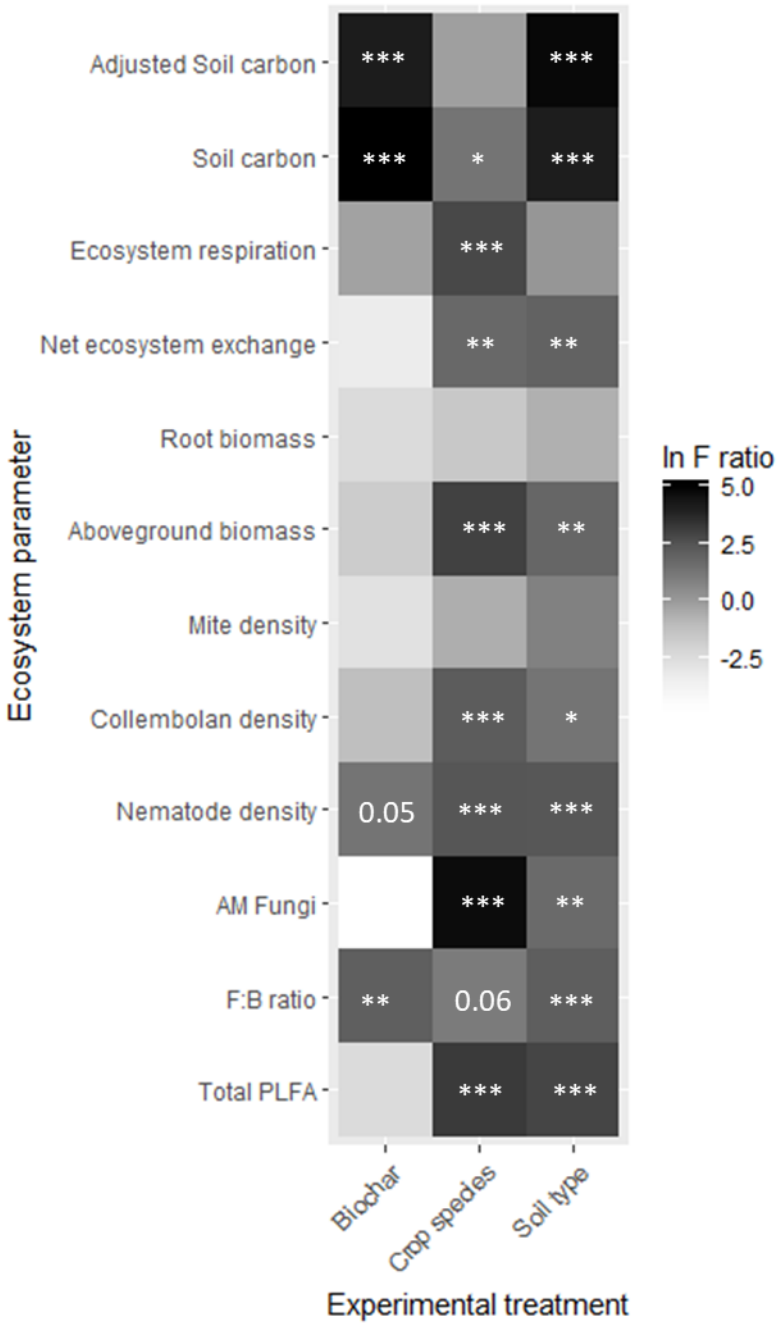

Adjusted Soil carbon
Soil carbon
Ecosystem respiration
Net ecosystem exchange
Root biomass
Aboveground biomass
Mite density
Collembolan density
Nematode density
AM Fungi
F:B ratio
Total PLFA
***
*
**
0.05
0.06
Ecosystem parameter
Biochar
Crop species
Soil type
Experimental treatment
ln F ratio
5.0
2.5
0.0
-2.5

Fig. 2

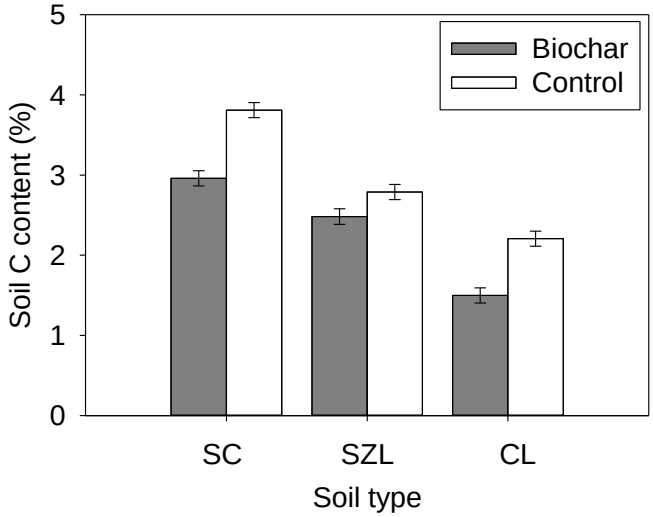


Fig. 3

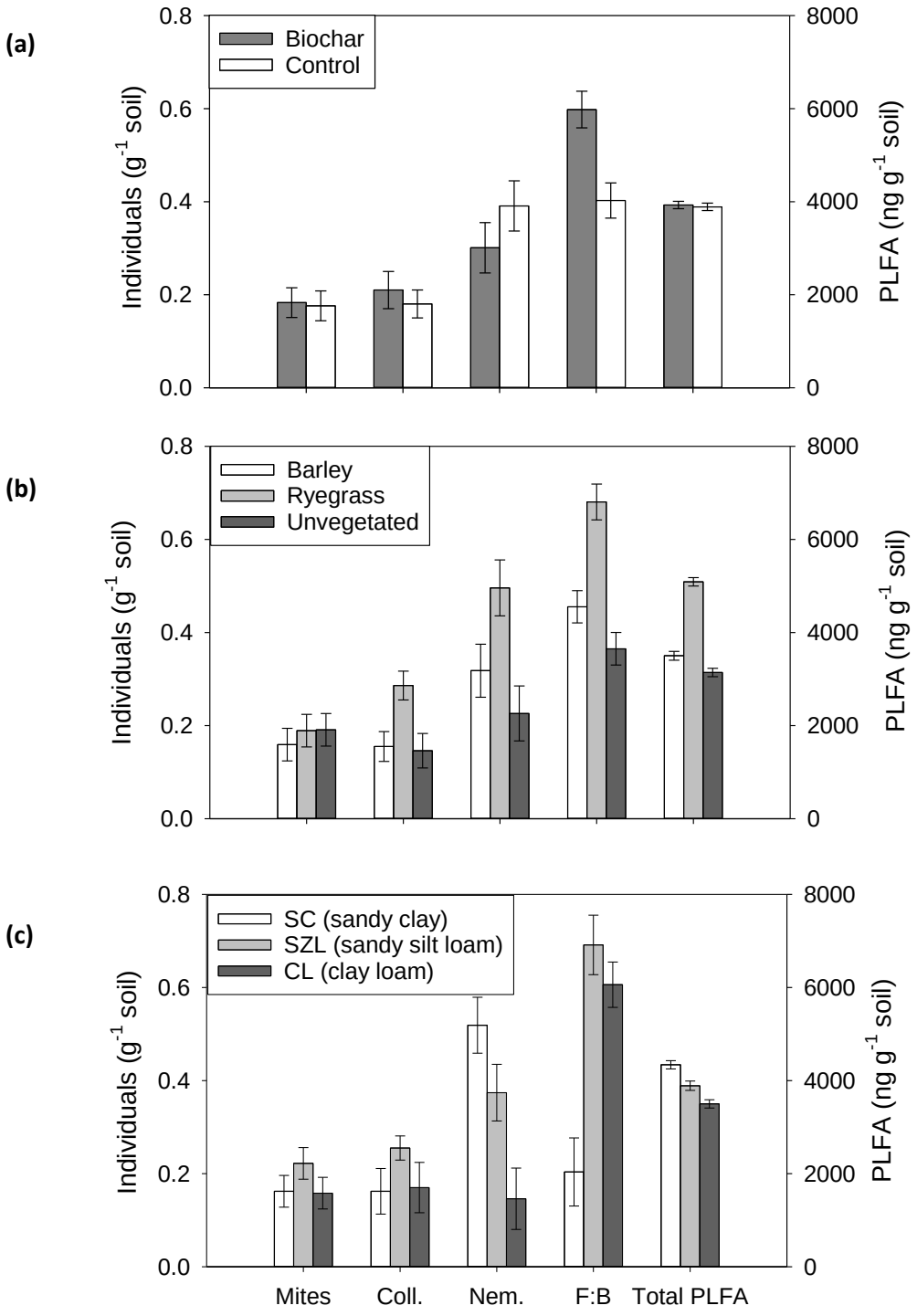
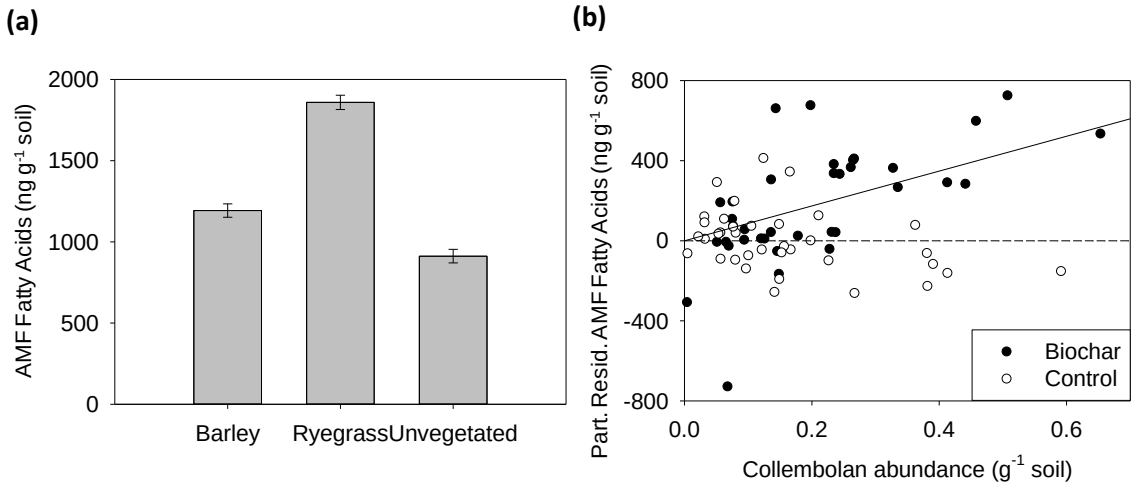

(a)
Biochar
Control
Individuals (g-1 soil)
PLFA (ng g-1 soil)
0.0
0.2
0.4
0.6
0.8
0
2000
4000
6000
8000
(b)
Barley
Ryegrass
Unvegetated
Individuals (g-1 soil)
PLFA (ng g-1 soil)
(c)
SC (sandy clay)
SZL (sandy silt loam)
CL (clay loam)
Individuals (g-1 soil)
PLFA (ng g-1 soil)
Mites
Coll.
Nem.
F:B
Total PLFA

Fig. 4


(a)
(b)
AMF Fatty Acids (ng g-1 soil)
2000
1500
1000
500
0
Barley
Ryegrass
Unvegetated
Part. Resid. AMF Fatty Acids (ng g-1 soil)
800
400
0
-400
-800
0.0
0.2
0.4
0.6
Collembolan abundance (g-1 soil)
Biochar
Control