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Biochar application differentially affects soil micro-, meso-macro-fauna and plant productivity within a nature restoration grassland

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ABSTRACT

Biochar is proposed as an option to sequester carbon (C) in soils and promote other soil-based ecosystem services. However, its impact on soil biota from micro to macroscale remains poorly understood. We investigated biochar effects on the soil biota across the soil food web, on plant community composition and on biomass production. We conducted a field experiment in a nature restoration grassland testing four treatments: two biochar types (herbaceous feedstock pyrolyzed at 400 °C or 600 °C – hereafter B400 and B600), and a positive (i.e. unpyrolysed biochar feedstock, hereafter Hay) and negative (no addition) control. Responses of plants and soil biota were evaluated one and three years after establishing the treatments.

Soil pH and K concentrations increased significantly in the B600 treatment. Mite abundances were significantly higher in B400 whereas nematode abundances were highest in Hay (1st year) and lowest in B400 (3rd year). Other soil fauna groups (enchytraeids and earthworms) varied more between years than between treatments. Legume cover increased significantly in the biochar treatments but this effect was transient. Legumes, grasses and primary productivity also showed a statistically significant Treatment x Year interaction due to transitory effects that were no longer present by the 3rd year.

Our results suggest that biochar produced from meadow cuttings and applied at the 10 t/ha rate cause transitory impacts on soil biota abundance and plant communities over the 3-year timeframe used for this experiment. Therefore, this type of biochar could potentially be used for soil carbon sequestration, with minimal impacts on soil biota abundance or diversity, within the groups studied here, or plant biodiversity and productivity. Further research is required to investigate the longer-term impacts of this potential soil C storage sink.

1. Introduction

Biochar, pyrolyzed biomass, is advocated as a means of concurrently sequestering C in soil while maintaining or enhancing the provision of other ecosystem services (Blanco-Canqui., 2021; Lehmann et al., 2006), such as crop productivity (Jeffery et al., 2017; Ye et al., 2020). Its properties are dependent on feedstock and production temperature (Ippolito et al., 2020). Investigation into the effects of biochar on soils has mainly focussed on changes in abiotic soil properties such as increased pH, water and nutrient retention, aggregation, permeability (e.g. Mukherjee and Lal, 2013; Herath et al., 2013) and effects on plant productivity (e.g. Liu et al., 2013; Jeffery et al., 2017; Ye et al., 2020). However, while some research has investigated biochar impacts on the soil microbiota such as fungi and bacteria (e.g. Lehmann et al., 2011; Xu et al., 2016), its impacts on soil microfauna and mesofauna, such as protozoa, nematodes and enchytraeids, and on larger animals as e.g.

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earthworms, remain little researched (Briones et al., 2020; Gruss et al., 2019; Llovet et al., 2021; Tammeorg et al., 2017). These soil organisms are of key importance for a range of ecosystem processes including organic matter decomposition, nutrient cycling, plant productivity, and soil structure formation (Bardgett, 2005; Nielson et al., 2011; Wardle et al., 2004). Therefore, it is important to understand the effects of biochar on these groups of soil organisms and their interactions with plant communities.

Biochar can influence soil organisms directly and indirectly, and positively or negatively, which can then feedback and affect plant communities (Wardle et al., 2004). Directly, biochar may provide food for soil organisms, for example through labile C on the char surface (Munksgaard et al., 2019). It can also create microhabitats in the inner pore space of biochar particles (Schnee et al., 2016; although debated, see Quilliam et al., 2013). Direct negative impacts can occur through toxic effects by compounds such as polyaromatic hydrocarbons (PAHs), which may be present in the charred material (Wang et al., 2018). However, PAH solubility is very low and so the prevalence of this issue is uncertain. Indirect effects following biochar application (McCormack et al., 2013) include effects on soil organisms via increased soil pH, reduced bulk density, higher soil moisture content, and altered nutrient adsorption capacity (Gul et al., 2015). These effects are dependent on application rates and biochar characteristics, which are determined by feedstock type and pyrolysis conditions (Llovet et al., 2021). These characteristics can also drive plant productivity and community effects (Jeffery et al., 2017; van de Voorde et al., 2014).

Our knowledge regarding the effects of biochar on soil biota is predominantly based on short-term (e.g. days-weeks) laboratory experiments using soil fauna model species, such as earthworms (Busch et al., 2011), collembola (Reibe et al., 2015) and enchytraeids (Marks et al., 2014). The outcomes of these studies are inconclusive, including both positive and negative impacts, and depend on biochar, soil type, and species tested, as well as on interactions with other organisms (Domene et al., 2015). For example, Briones et al. (2020) showed that biochar additions to a commercial *Miscanthus* bioenergy plantation had a negative effect on abundance and species richness of larger-sized soil fauna such as earthworms, whereas mesofauna (enchytraeids, mites and collembola) abundance and diversity increased following biochar addition. Therefore, results from biochar experiments using individual faunal species in short-term incubations may differ from those under field conditions using intact soil food webs and including (semi)natural vegetation.

In this field study, we examined the effect of soil amendments (herbaceous feedstock and two types of biochar) on the abundance and composition of soil biota across different trophic levels of the soil foodweb in a grassland restoration experiment. The two biochars were produced at different temperatures (400 °C and 600 °C; hereafter B400 and B600) using the same feedstock, (Gundale and DeLuca, 2006; Kasozi et al., 2010; Spokas, 2010; Zhao et al., 2013). This was done as evidence suggest that biochars produced at temperatures >500 °C are expected to be more recalcitrant than those produced at lower temperatures (Ippolito et al., 2020). The unprocessed residue from which the biochar was produced (hereafter "Hay") was included as a positive control (Jeffery et al., 2015), and which can be expected to function as a substrate for the soil biota. A negative control was included which comprised no organic material addition to the soil.

We hypothesised that:

(H1). the effect on abundance of soil organisms will decrease with increasing recalcitrance of the substrate (i.e. effect size: Hay > B400 > B600 as high-temperature biochars are more recalcitrant to degradation).

(H2). these effects will differ between different groups of soil organisms.

(H3). there will be a significant effect on plant productivity

(H4). these effects will differ between different plant groups

(H5). impacts will decrease leading to a reduction in observed treatment effects over time.

2. Materials and methods

2.1. Experimental field site

We established an experimental field site in April 2011 in a nature restoration area in the Veluwe in the Netherlands (52°04′ N, 05°45' E) - agriculture was abandoned at the site in 1996. The experiment is described in detail in van de Voorde et al. (2014). In short, the field experiment consisted of four treatments and six replicate blocks, set up in a randomized complete block design, resulting in 24, 4 × 4 m, plots. The four treatments were: incorporation of biochar produced at 400 °C (B400), biochar produced at 600 °C (B600), incorporation of dried non-pyrolyzed cuttings from which the biochar was produced (Hay), and a control treatment in which no material was incorporated (Control). Chemical characteristics are included in Table 1. The soil contained 93.9% sand, 5.3% silt, 3.4% clay, with a soil organic C of 2.8%, Mineral N 6.9 mg/kg, P-PO 44.4 mg/kg, K 46.2 mg/kg, soil pH_{KCl} 4.96.

Feedstock for the biochar consisted of cuttings from the experimental site, collected in October 2010. The cuttings were air dried, shredded and homogenized. One third of the cuttings were then pyrolyzed at 400 °C and another third at 600 °C (Biogreen, ETIA, France). The final third was left unpyrolyzed, and applied as hay residue. Biochar and the hay residue (Hay) were applied at a rate of 1.3 kg/m^2, corresponding to an application rate of 1% (m/m) equivalent to ~10 Mg/ha. After applying the amendments to the surface, the top ~15 cm of all plots (including Control) were cultivated using a rotavator and sown with a mixture of 18 grassland species totalling ~5000 seeds/m^2 (see mixture in van de Voorde et al., 2014). Each October, the field site was mown and all aboveground biomass removed aimed at impoverishing the site to help it return from current state to a high biodiversity grassland, as described in van de Voorde et al. (2014).

2.2. Sampling and identification of soil organisms

In August 2011 and 2013, we took soil samples using specific methods for each organism group (see details in Supplementary Information) from randomly selected points within the inner 3 × 3 m of each plot (i.e. leaving a 1 m boundary around the edge of each plot to minimise edge effects). In each plot, we took four soil samples: 1) a bulk soil sample consisting of nine soil cores (0–15 cm, 3 cm diameter, sieved through a 1 cm mesh) used for the extraction of nematodes and protozoa, measurement of fungal and bacterial biomass, and nutrient analyses; 2) an intact soil core (0–10 cm, 10 cm diameter) for the extraction of microarthropods (mites and collembola; 3) an intact soil core (0–5 cm, 10 cm diameter in 2011 and 5 cm in 2013) for the extraction of enchytraeids; and 4) a 15 × 15 × 15 cm soil monolith was excavated to collect macroarthropods (i.e. all soil dwelling arthropods >2 mm in size - hereafter Macroarthropods) and earthworms. All samples were stored

Table 1
Chemical characteristics of the two biochar types and the feedstock from which it was produced (Hay). Values show means ± standard error (n = 3). Adapted from van de Voorde et al. (2014).

	B400	B600	Hay
Total C (%)	41.8 ± 0.3	57.7 ± 1.7	41.7 ± 0.3
Total N (%)	1.67 ± 0.05	2.05 ± 0.03	1.49 ± 0.06
C:N	25.2 ± 0.6	28.2 ± 1.2	28.1 ± 1.0
pH_{H2O}	8.5 ± 0.03	9.82 ± 0.03	6.09 ± 0.01
Mineral N (mg/kg)	0.8 ± 0.03	0.8 ± 0.06	–
P-PO_4 (mg/kg)	1.9 ± 0.02	0.6 ± 0.03	–
K (mg/kg)	1620.8 ± 24.2	1684.8 ± 28.5	–

at 5 °C until analyses. All organisms were counted and identified to species, genus or group level (in case of protozoa). Numbers of individuals were then calculated to numbers per 100 g dry weight soil.

Plant species cover was recorded as percentage on a continuous scale by visual estimations in two 1 × 1 m quadrats. Aboveground biomass was collected in two 25 × 25 cm quadrats per plot. Plant material was dried at 70 °C for five days before estimation of dry weight.

Bacteria and fungi: Phospholipid fatty acids (PLFA) were extracted from freeze-dried soil samples (approximately 3 g dry weight) with a three-step extraction protocol (Boschker, 2004). This consisted of a Bligh and Dyer total lipid extraction, a fractioning of the total lipids on a silicic-acid column with chloroform, acetone and methanol, and a mild-alkaline derivation to methylate to fatty acid methyl esters (FAMEs). The FAMEs were analysed using gas chromatography–flame ionization detector (GC–FID) on a Focus GC (Thermo Scientific, Bremen, Germany) with a Zebron ZB5 column (dimensions: 60 m, 0.32 mm, 0.25 lm; Phenomenex, Torrance, CA, USA) in 2011 and on an Agilent technologies 7890A GC-FID with a HP-5MS column (dimensions: 60 m, diameter 0.250 mm, film 0.25 μm Agilent technologies Santa Clara, CA, USA) in 2013. The data from the two different machines are not directly comparable, and hence the two years were analysed separately. Peak areas were calculated relative to the internal standard 19:0. The PLFAs c14, i-C15:0, ai-C15:0, C15:0

i-C16:0/C16:4ω3, C16:1ω7c, C16:1ω7t/C16:2ω4, C16:1ω5c + t, C16:0, 10Me–C16:0, i-C17:0, ai-C17:0, C17:1ω8, c-C17:0, c17:0, 10Me–C17:0, C18:2ω6c, C18:1ω9c/2ω6t/3ω3, C18:1ω7c/C18:1ω9t, C18:0, cy19:0. Of these, i-C15:0, a15:0, 15:0, i16:0, 16:1ω7t, a17:0, 17:0, cy17:0, 18:1ω7 and cy19:0 were used to represent bacterial biomass (Frostegård and Bååth, 1996), 18:2ω6 for total fungal biomass (Klamer and Bååth, 2004).

Protozoa: We estimated the number of protozoa, i.e. naked amoebae and heterotrophic flagellates, using a most probable number method (Rønn et al., 1995). From each of the 24 plots we mixed two samples of 5 g (fresh weight) soil with 100 ml of 1/300 TSB growth medium (0.1 g L^{-1} autoclaved solution of Tryptic Soy Broth; Difco Bacto®), homogenized the mixture for 1 min in a blender, and prepared eight serial three-fold dilution of 12 dilutions in microtiter plates using 1/300 TSB solution as growth medium. The plates were stored in the dark at 11 °C and the number of protozoa, distributed on naked amoebae and heterotrophic flagellates, were scored after one and three weeks of incubation.

Enchytraeids: Enchytraeids were extracted from the intact soil cores using a modified wet funnel method (O'connor, 1955), collected alive and then fixed in 70% EtOH and their abundance quantified using a microscope.

Nematodes: Nematodes were extracted from 100 g of soil using Oostenbrink elutriators (Oostenbrink, 1960). Nematodes were heat-killed and fixed (35% formaldehyde diluted to 4%). Total number of nematodes was counted and nematodes were identified in 10% of the total extract. Nematodes were identified to genus or family level, according to Bongers and Bongers (1998), and allocated to functional groups (plant, bacterial, fungal feeders and omnivores) according to Yeates et al. (1993).

Microarthropods: Collembola and mites, were extracted from intact soil cores using Tullgren extraction funnels for 7 days (Macfadyen, 1961). After extraction, the cores were dried for 3 days at 70 °C to determine their dry weight. Animals were collected in 70% EtOH, counted using a microscope, and identified to species level. Mites were further grouped into *Mesostigmata* and suborders: *Prostigmata*, *Astigmata*, and *Oribatida*.

Earthworms and Macroarthropods: Earthworms (Lumbricidae) and Macroarthropods were hand-sorted from the excavated soil monoliths and counted at the time of collection. Arthropods were stored in 70% EtOH until identification. Earthworms were stored on moist paper at 20 °C for 2 days before they were grouped into ecological categories (i.e. to epigeic, endogeic and anecic groups) and weighed.

2.3. Soil and biochar nutrient analyses

$N–NO_3$, $N–NH_4$, $P-PO_4$ and K were determined photometrically (540 nm) in a 1:10 (w/v) 0.01 M $CaCl_2$ extract using an auto-analyser (Skalar, the Netherlands). This was also done for the biochar, but not for the plant materials (Hay) as this approach measures available rather than total nutrients, which are negligible from undecomposed plant material. pH was measured in a 1:5 (v/v) aqueous solution. Moisture content of the soil was determined gravimetrically (105 °C, 48 h). Organic matter content was determined by loss on ignition (550 °C, 3 h).

2.4. Data analyses

Abundance data for different groups of soil organisms were analysed treatment and year as fixed effects and block as a random effect, using a linear model approach following established methods (Zuur et al., 2009). Assumptions of residual distribution, homoscedasticity and independence were examined and met for all analyses. Where transformation was necessary to conform to linear model assumptions, it is indicated (Table S2).

Multivariate analysis was performed to look at the contribution of individual PLFAs to overall PLFA content variation within and between treatments, and similarly for contribution of different taxonomic groups to overall abundance variation within and between treatments. We did this using Principal Components Analysis (PCA), following established methods (Borcard et al., 2011). Briefly, for PCA analyses individual variables were centred and scaled and the results interpreted based on the variables with the highest ranked loadings associated with the first two axes of variation. PCA of the PLFA data was performed separately for Year 1 and Year 3 of the experiment for the organismal abundance data to examine changes in organismal abundance over time.

All analyses were performed in R version 4.03 (R Core Team, 2020).

3. Results

3.1. Soil nutrients and pH

The concentration of K was significantly higher in both biochar treatments than the Control in both 2011 and 2013 ($P < 0.01$; Fig. 1a). There was no significant difference in K concentration between years ($P = 0.46$), nor was there a significant interaction between treatment and year ($P = 0.98$) Soil pH was overall significantly higher in the B600 treatment than the Control ($P < 0.01$), and was significantly lower, by an average 0.2 units, in 2013 than in 2011 ($P < 0.01$). However, the interaction between treatment and year was not significant. Neither soil mineral N nor PO_4 levels differed significantly between treatments ($P = 0.83$ and $P = 0.12$; Fig. 1b & d respectively) but both were significantly higher in 2013 than in 2011 ($P < 0.01$ for both nutrients).

3.2. Plant communities

The total productivity of the plant communities was significantly lower in 2013 than in 2011 by, on average, 44% ($P < 0.01$; Fig. 2a). The cover of grasses increased significantly, approximately 8-fold on average ($P < 0.01$; Fig. 2b) whereas the cover of legumes and forbs was significantly lower in 2013 by 89% and 52% respectively ($P < 0.01$; Fig. 2 c & d), whereas. A significant Treatment × Year interaction was observed for total productivity ($P = 0.02$), percent grass cover ($P = 0.04$) and percent legume cover ($P = 0.01$).

Legume cover was significantly higher in plots that received biochar (B400 and B600) compared to both the Hay and Control treatments in 2011 ($P < 0.01$; Fig. 2c). However, this effect was no longer present in 2013, with a significant difference between years ($P < 0.01$) and a significant Treatment × Year interaction ($P < 0.01$).

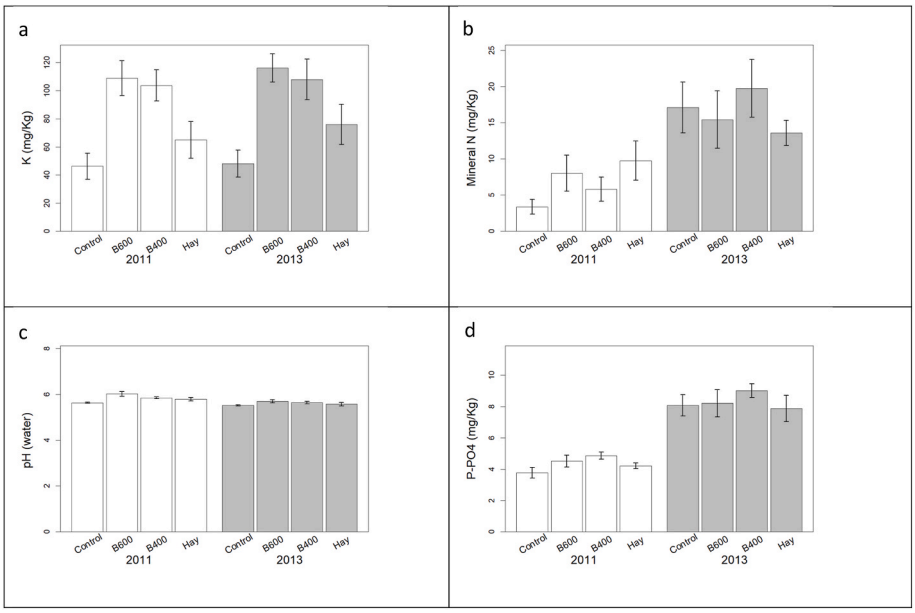

Fig. 1. Mean (±1 SE) available soil nutrient content and pH-H_2O in relation to the four treatments, Control, B600, B400, Hay, in 2011 (white bars) and 2013 (shaded bars). Mineral N is the sum of N–NO_3 and N–NH_4^+.

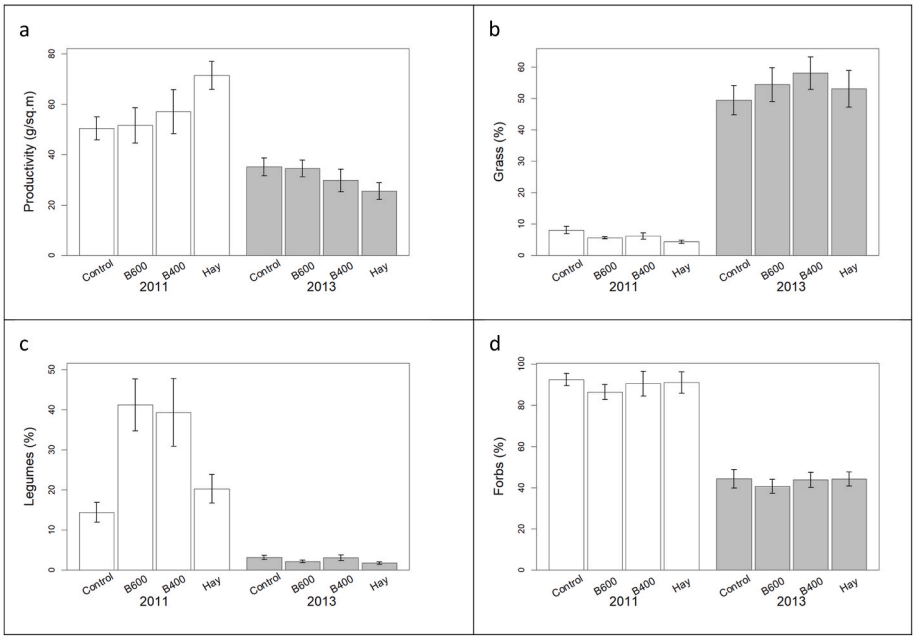

Fig. 2. Mean (±1 SE) aboveground productivity and percentage cover per plant functional group in relation to the four treatments, Control, B600, B400, Hay, in 2011 (white bars) and 2013 (shaded bars).

3.3. Soil organisms

There was no significant impact of Biochar or Hay between treatments (P = 0.32) or across years (P = 0.37; Fig. 3a). Earthworm abundance was not also significantly different between treatments (P = 0.74) nor between years (P = 0.67; Fig. 3b). There were no significant interactions between treatment and year for any of the ecological earthworm groups investigated (Table S1).

Enchytraeid abundance was not significantly affected by treatment (P = 0.1) but showed significant differences between years; a reduction in their abundances was observed in 2013 across all treatments (P < 0.001; Fig. 3c).

Macroarthropods showed no significant treatments effects (P = 0.82) or but had significantly higher abundances across all treatments in 2013 than 2011s (P = 0.00.02; Fig. 3d).

Mite abundances were not significantly affected by treatment for any of the groups investigated (Fig. 3e). However, there were significant differences between years for Mesostigmata (P = 0.003), Oribatida (P = 0.001) and Prostigmata (P = 0.014). Astigmata abundances were not significantly different across years (P = 0.35) (Fig. 4a–d).

Nematodes were significantly more numerous in the Hay treatment in 2011 (P = 0.004) and differed significantly between years (P = 0.003) (Fig. 3f). No significant treatment effect on nematode abundance was observed in 2013 – the third year of the experiment, and the interaction between treatment and year was not significant (P = 0.16) (Table 2). The abundances of all nematode groups differed significantly between

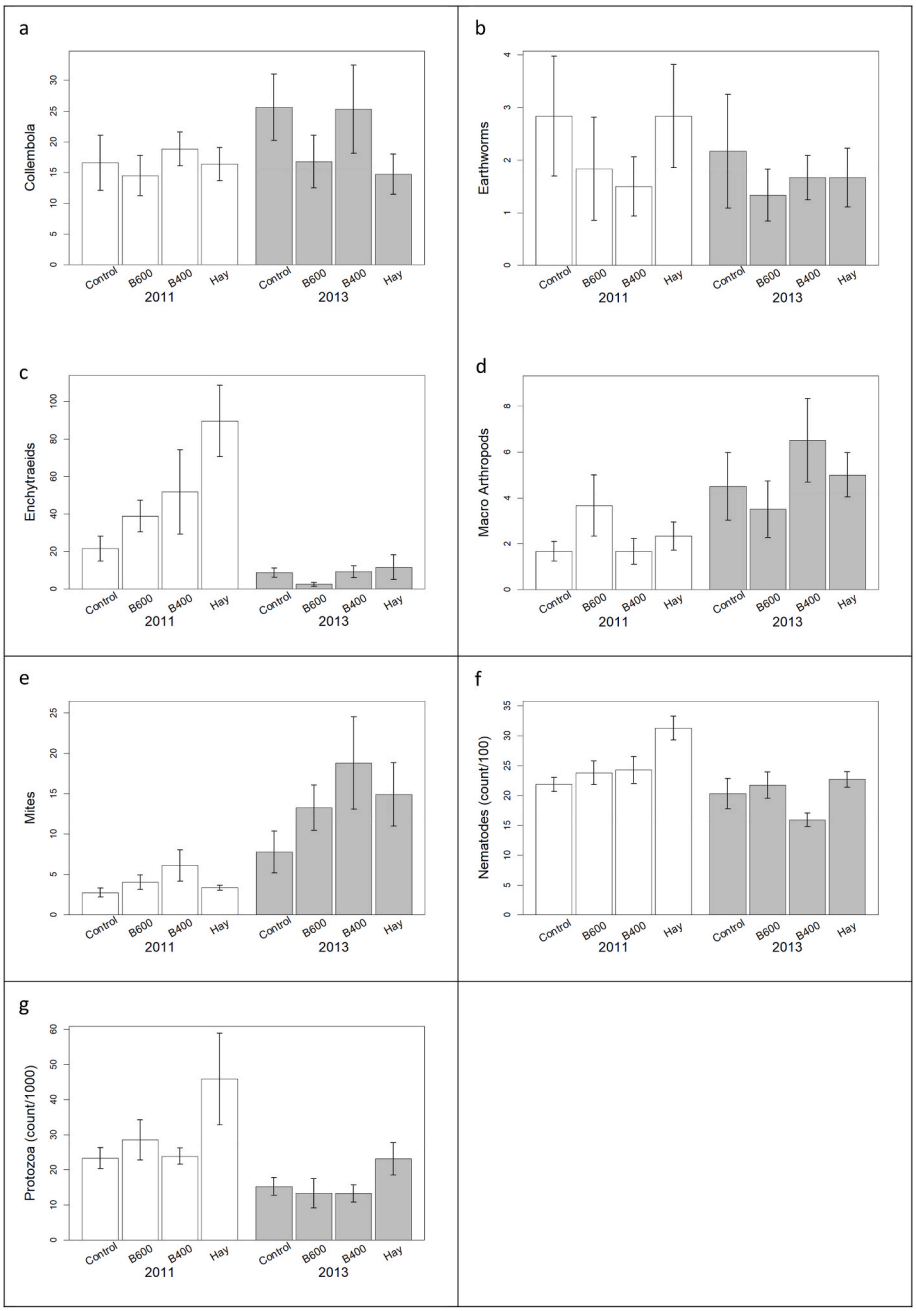

Fig. 3. Mean (±1 SE) abundance of broad groups of soil organisms (individuals/00 g dw soil for all organisms except earthworms which are reported as g m^{-2}) in relation to the four treatments, Control, B600, B400, Hay, in 2011 (white bars) and 2013 (shaded bars).

years ($P < 0.01$), except for the plant feeders ($P = 0.32$) (Fig. 4e–h). There was no significant interaction between treatment and year for any of the nematode groups (Table S1).

The Protozoan numbers were significantly affected by treatment ($P < 0.001$) with higher abundances recorded in the Hay treatment than in the other treatments (Fig. 3g). The abundance of both amoebae and flagellates varied significantly across years ($P = 0.026$ and $P < 0.001$ respectively) (Fig. 4 l&m).

Principal component analysis of the abundances of the belowground communities suggested that there was discrimination in PC1 (32.3%) between the Hay treatment and the Control and two biochar treatments (Fig. 5a). There was little to no discrimination between the treatments in PC2 (16.7%), as evidenced by the largely overlapping error bars. The main groups responsible for the observed discrimination in PC1 were bacteria feeding nematodes, followed by fungal feeding nematodes and enchytraeids. There was no evidence for discrimination between any of the treatments and control from the samples collected in 2013 (Fig. 5b).

The PCA analysis of the microbial communities, as indicated by PLFA composition, showed that the Hay treatment differed to those present in the two biochar and control treatments in 2011 (Fig. 6a). Discrimination mainly occurred in PC1, which accounted for 27.6% of the total variation. No discrimination was apparent between B400, B600 and Control. And no discrimination between any of the treatments, in either PC, was apparent in analysis for 2013 (Fig. 6b). There was no significant difference in bacterial:fungal ratio between treatments in either 2011 ($P = 0.16$) or 2013 ($P = 0.06$).

4. Discussion

Biochar application to soil resulted in a range of significant

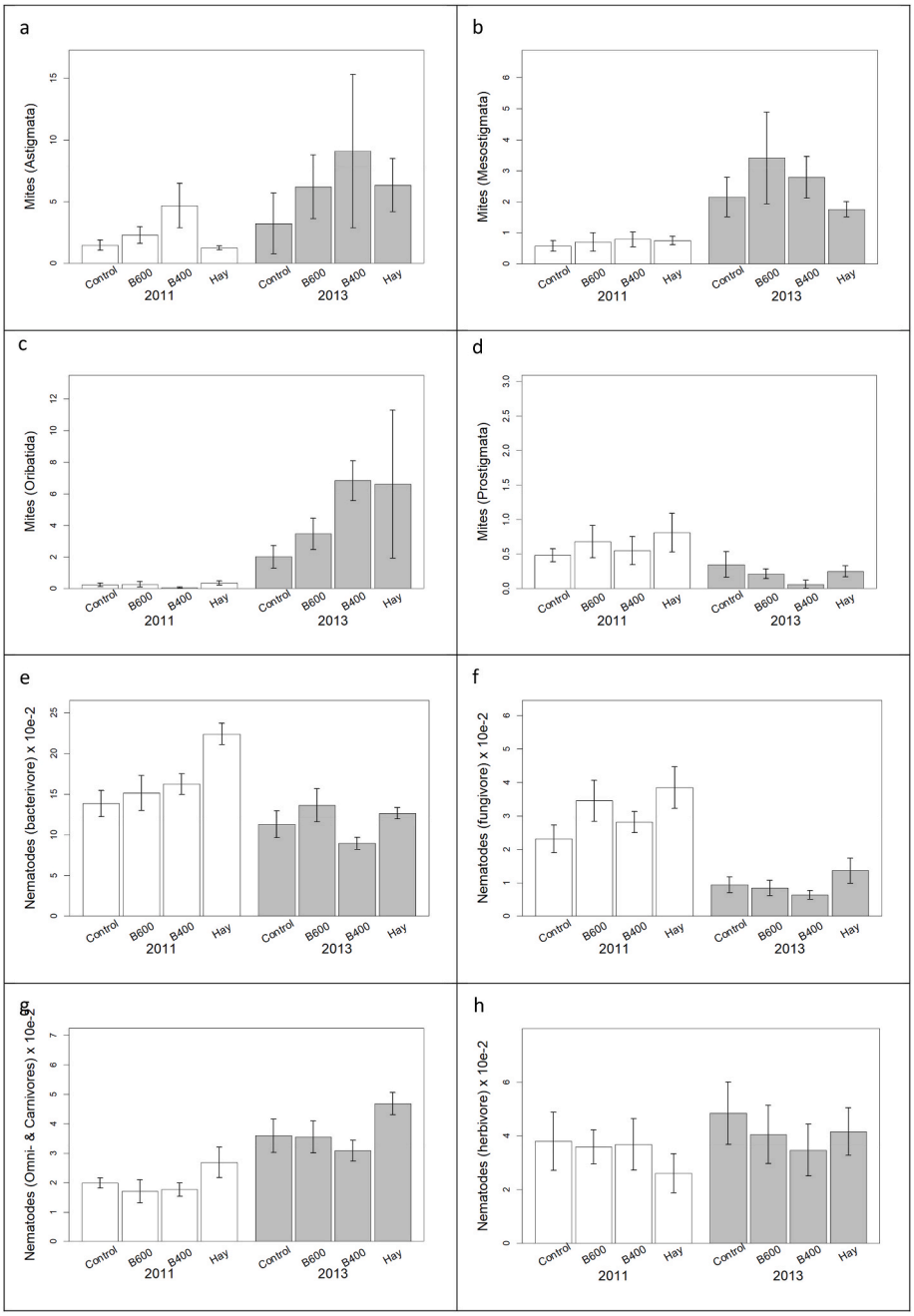

Fig. 4. Mean (±1 SE) abundance of soil organism sub-groups (number of organisms/00 g dw soil) in relation to the four treatments, Control, B600, B400, Hay, in 2011 (white bars) and 2013 (shaded bars).

physicochemical and biological effects. The magnitude of the effect varied between groups. Legumes increased in response to biochar addition whereas belowground groups responded more to the input of Hay, which likely functioned as a substrate and stimulated growth. While the addition of the Hay increased abundances of some soil organisms there was no clear difference between the biochar types. Therefore, H1 should be rejected. However, it is possible that the two biochars used in this experiment were not sufficiently different in recalcitrance for any effect to be picked up over the timeframe of this experiment.

4.1. Soil properties

Soil potassium concentration in the soil increased significantly in both biochar treatments, likely because the ash portion of the biochar was rich in potassium (Oram et al., 2014). The pH of both biochar treatments also increased significantly, by 0.2 pH units for B600 and by 0.1 pH units for B400. This is in line with the high pH of the biochar used (van de Voorde et al., 2014), and is expected to occur with most other biochar applications to soil too, due to the high pH of biochar (Fidel et al., 2017; Jeffery et al., 2017).

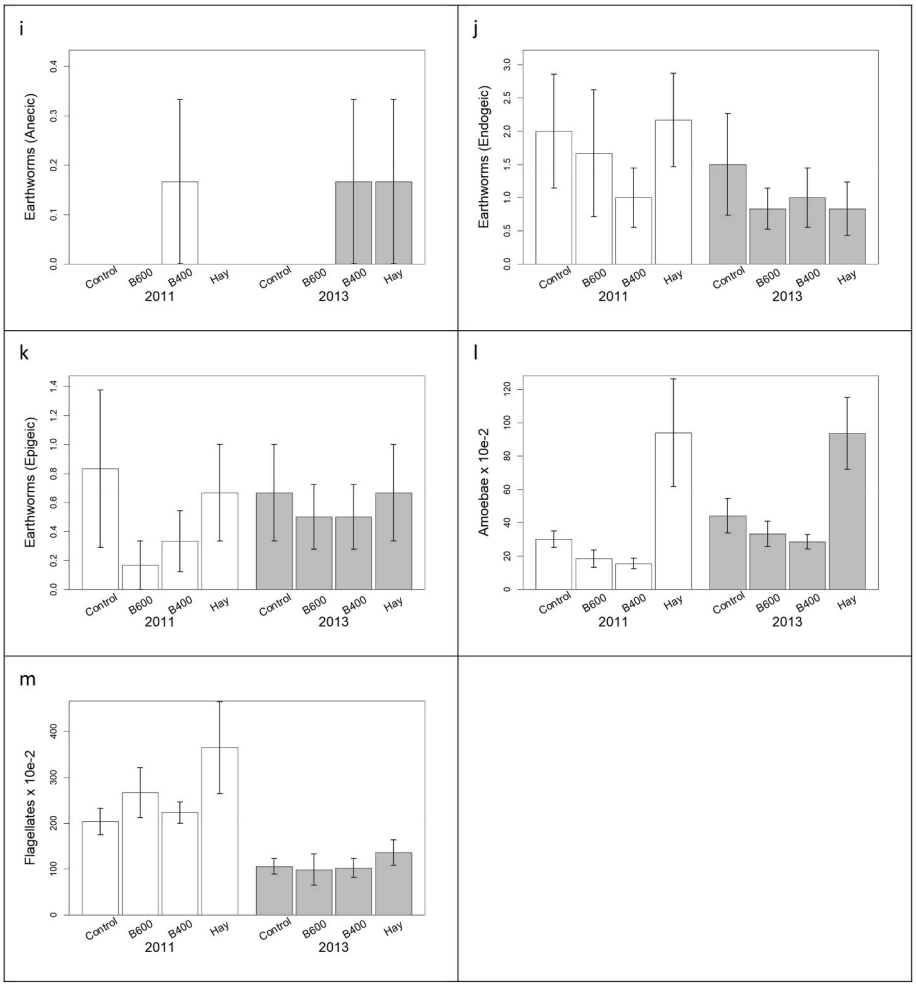

Fig. 4. (*continued*).

4.2. Soil organisms

The soil organism groups responded differently to the treatments and their response varied between years. The effect on the abundance of soil organisms appeared to be related to the level of recalcitrance of the material added (i.e. Hay <. B400 < B600; H1). However, no significant differences were observed for the two biochar types used in this study. We hypothesised that the biochar produced at higher temperature would be more recalcitrant based on, for example, Budai et al. (2016). It is possible, though, that the difference in production temperature was not sufficient to drive differences in recalcitrance.

Hay addition (H2) stimulated growth in some groups more than others. Significant increases in abundances were observed in bacteria (indicated by increased abundance of bacterial-feeding nematodes, although not reflected in the bacterial: fungal PLFA ratios, possibly due to fungi also increasing in biomass due to addition of the hay), enchytraeids and protozoa. Observed impacts decreased between sampling times, meaning that H5, i.e. impacts will decrease with time, can be accepted for soil organisms, although with caveats as detailed below.

Earthworm abundance did not vary significantly between treatments or across years (Fig. 3), possibly because we used a lower application rate (10 t/ha) than others who did report an effect (e.g. Briones et al., 2020; Llovet et al., 2021) also reported a decrease in earthworm biomass and abundance with increased biochar addition rates. This discrepancy between our findings and these other studies may be due to the different soil or biochar types used, or more likely is due to the lower application rates used in this experiment (i.e. 10 t/ha).

In our experiment, mites and Macroarthropods (Fig. 3) were more abundant across treatments in 2013 than 2011. This may be due to legacy effects of the establishment of the plots, notably the ploughing and rotovating as part of experimental set up. Physical disturbance disrupts the existing soil communities. In particular, it affects those feeding on fungi as fungal hyphal networks are disrupted more and take longer to recover than bacterial communities. This finding agrees with work by Bedano et al. (2006) and Callaham et al. (2006), both of which showed delayed effects beyond the first season for micro and macroathropods.

Conversely, enchytraeids and protozoa abundances were higher in 2011 than 2013 across all treatments, which may have been due to increased substrate availability following destruction of soil aggregates after ploughing (Zheng et al., 2018) which increased bacterial access to previously protected SOM (Rabbi et al., 2016). Increased bacterial diversity and growth could then have promoted bacterial grazers such as protozoa, nematodes and enchytraeids (e.g. Cambardella and Elliott, 1994). Furthermore, these two groups were most abundant in the Hay treatment in 2011, likely due to the increased substrate availability provided by addition of this new residue. This is evidence that supports H2 and was expected as many enchytraeids (Graefe et al., 1999) and protozoa species (Laybourn-Parry, 1984) respond rapidly to the addition of a new food sources.

With respect to the microbial communities (as indicated by on PLFA profiles) some discrimination between Hay and Control was observed in PC1 (Fig. 6) as was hypothesised (H1). However, while the two biochar treatments B600 and B400 fall between Hay and Control, they do not

Table 2
P-values for main effects of treatment, year and treatment x year interaction on the measured variables. All models use block as a random effect. All models that did not fit assumption of Gaussian residual error and homoscedasticity, were log transformed.

Measure (transformation)	Treatment P-value	Year P-value	Treatment x Year P-value
K (mg/kg)	< **0.001**	0.46	0.98
Mineral N (mg/kg)	0.83	< **0.001**	0.19
$pH_{(H2O)}$	< **0.001**	< **0.001**	0.32
P-PO_4 (mg/kg)	0.12	< **0.001**	0.85
Productivity (g/m^2) (log)	0.96	< **0.001**	**0.023**
Grasses (%) (log)	0.11	< **0.001**	**0.044**
Legumes (%)	**0.042**	< **0.001**	**0.011**
Forbs (%)	0.43	< **0.001**	0.98
Macroarthropods (log+1)	0.82	**0.002**	0.20
Earthworms (log+1)	0.74	0.67	0.86
Enchytraeids (log+1)	0.10	< **0.001**	0.07
Mites (log)	**0.042**	**0.002**	0.87
Collembola (log)	0.32	0.37	0.51
Nematodes (log)	**0.004**	**0.003**	0.16
Protozoa (log)	0.073	**0.002**	0.77

Measure (transformation)	Treatment P-value	Year P-value	Treatment x Year P-value
Earthworms (log+1)	0.74	0.67	0.86
Enchytraeids (log+1)	0.10	< **0.001**	0.07
Nematodes: bacteria (log)	**0.016**	< **0.001**	0.10
Nematodes: fungal (log)	0.08	< **0.001**	0.62
Nematodes: omnivore (log)	**0.034**	< **0.001**	0.80
Nematodes: plant (log)	0.73	0.32	0.58
Mites: Astigmata (log+1)	0.46	0.35	0.73
Mites: Mesostigmata (log+1)	0.72	**0.0032**	0.70
Mites: Orbatida (log+1)	0.21	**0.0013**	0.058
Mites: Prostigmata (log+1)	0.52	**0.014**	0.72
Earthworms: Anecic	-	-	–
Earthworms: Endogeic (log+1)	0.80	0.24	0.76
Earthworms: Epigeic (log+1)	0.68	0.51	0.91
Amoebae (log)	< **0.001**	**0.026**	0.75
Flagellates (log)	0.37	< **0.001**	0.74

Year	Measure (Count)	Control Mean (SE)	B600 Mean (SE)	B400 Mean (SE)	Hay Mean (SE)
2011	Nematodes: Bacterivore	1383.3 (161.7)	1512.2 (215.7)	1623.7 (127.8)	2238.2 (131.2)
	Nematodes: Fungivore	231.0 (41.1)	344.8 (61.0)	281.0 (32.0)	384.3 (61.6)
	Nematodes: Omnivore	197.9 (17.1)	169.9 (39.3)	175.8 (22.4)	268.0 (51.9)
	Nematodes: Herbivore	379.8 (108.2)	358.8 (63.7)	367.9 (95.7)	259.8 (72.5)
	Mites: Astigmata	1.5 (0.4)	2.3 (0.7)	4.7 (1.8)	1.3 (0.2)
	Mites: Mesostigmata	0.6 (0.2)	0.7 (0.3)	0.8 (0.2)	0.8 (0.1)
	Mites: Orbatida	0.2 (0.1)	0.3 (0.2)	0.1 (0.0)	0.3 (0.1)
	Mites: Prostigmata	0.5 (0.1)	0.7 (0.2)	0.5 (0.2)	0.8 (0.3)
	Earthworms: Anecic	0.0 (0.0)	0.0 (0.0)	0.2 (0.2)	0.0 (0.0)
	Earthworms: Endogeic	2.0 (0.9)	1.7 (1.0)	1.0 (0.4)	2.2 (0.7)
	Earthworms: Epigeic	0.8 (0.5)	0.2 (0.2)	0.3 (0.2)	0.7 (0.3)
	Amoebae	3015.0 (498.2)	1847.7 (511.3)	1549.4 (313.8)	9395.4 (3235.9)
	Flagellates	20353.7 (2874.1)	26665.4 (5464.4)	22326.1 (2332.9)	36458.7 (10010.4)
2013	Nematodes: Bacterivore	1128.0 (165.4)	1362.1 (204.0)	894.5 (72.7)	1262.7 (69.1)
	Nematodes: Fungivore	93.9 (23.6)	84.3 (22.4)	63.4 (12.8)	136.1 (37.3)
	Nematodes: Omnivore	359.1 (56.7)	354.8 (54.2)	308.2 (35.8)	468.2 (37.9)
	Nematodes: Herbivore	483.9 (115.3)	404.9 (108.8)	346.6 (96.3)	415.6 (88.2)
	Mites: Astigmata	3.2 (2.5)	6.2 (2.6)	9.1 (6.2)	6.3 (2.2)
	Mites: Mesostigmata	2.2 (0.6)	3.4 (1.5)	2.8 (0.7)	1.8 (0.2)
	Mites: Orbatida	2.0 (0.7)	3.5 (1.0)	6.8 (1.3)	6.6 (4.7)
	Mites: Prostigmata	0.3 (0.2)	0.2 (0.1)	0.1 (0.1)	0.2 (0.1)
	Earthworms: Anecic	0.0 (0.0)	0.0 (0.0)	0.2 (0.2)	0.2 (0.2)
	Earthworms: Endogeic	1.5 (0.8)	0.8 (0.3)	1.0 (0.4)	0.8 (0.4)
	Earthworms: Epigeic	0.7 (0.3)	0.5 (0.2)	0.5 (0.2)	0.7 (0.3)
	Amoebae	4416.9 (1039.6)	3342.1 (756.1)	2861.6 (427.9)	9353.9 (2153)
	Flagellates	10587.3 (1677.2)	9883.8 (3418.8)	10234.3 (2035.9)	13595.2 (2764.4)

S1. P-values for main effects of treatment, year and treatment xyear interaction are in cells. All models use block as a random effect. All models fit assumption of Gaussian residual error and homoscedasticity (transformation of dependent variable indicated where applied).
S2. Summary statistics for soil organisms (Corresponds to Fig. 4).

discriminate as hypothesised (i.e. Hay > B400 > B600 > Control). Biochar quality differences were hypothesised because B400 is expected to have more labile C present than B600 biochar (Enders et al., 2012; Zhao et al., 2013). Nevertheless, as variation of PLFA profiles within treatments was as great as variation between treatments, care must be taken not to overinterpret these data – spatial variability of microbial communities across the experimental site contributed to much of the variation observed.

Principal Component Analysis based on all soil groups analysed showed that the overall soil community composition responded to the addition of the Hay, discriminating this treatment from the other communities in PC1 in 2011 (Fig. 5a). This likely occurred as a response to increased substrate availability. Soil communities in plots amended with biochar did not discriminate from Control in either PC1 or PC2, suggesting that biochar did not significantly affect the belowground community either directly (i.e. as a substrate) or indirectly (through changes in the soil environment such as pH) (Zhao et al., 2013). There was no longer any discrimination between plots by 2013 (Fig. 5b). This further supports the hypothesis that initially observed impacts were due to the residue applied in the Hay treatment functioning as a substrate for the belowground community. That substrate – or at least the labile parts of it – had likely already been decomposed by 2013, meaning discrimination between Hay and the other treatments was no longer observed then. This finding disagrees with, for example, Marks et al. (2014), Domene et al. (2015), Schnee et al. (2016), Munksgaard et al. (2019) among others, who demonstrated in laboratory studies that biochar affects soil organisms. This difference in result may be due to the highly controlled nature of their experiments, without the impact of weather, vegetation and more complex soil communities present in field studies such as presented here. The biochars used in those studies were also produced from different feedstocks, which may also have contributed to differences between studies. Impacts on both plant and belowground communities and plant productivity are transient within the soils used for this field experiment. Further work is needed across a range of more nutrient and substrate limited soils, as well as with biochar with different characteristics, to confirm the generality of these results.

4.3. Plant community

Plant community composition is a strong driver of soil community

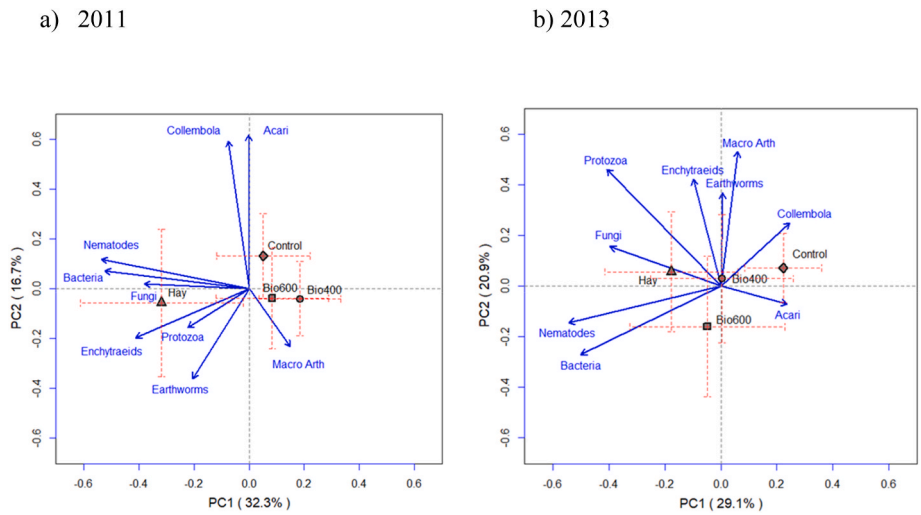

Fig. 5. Principal Component Analysis for soil organisms based on measures of nine taxonomic groupings. The eigenvectors for each PC axis are shown in blue. The treatment mean eigenvalues are shown with error bars (±1 standard deviation in red). Analyses are shown separately for 2011 (a) and 2013 (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

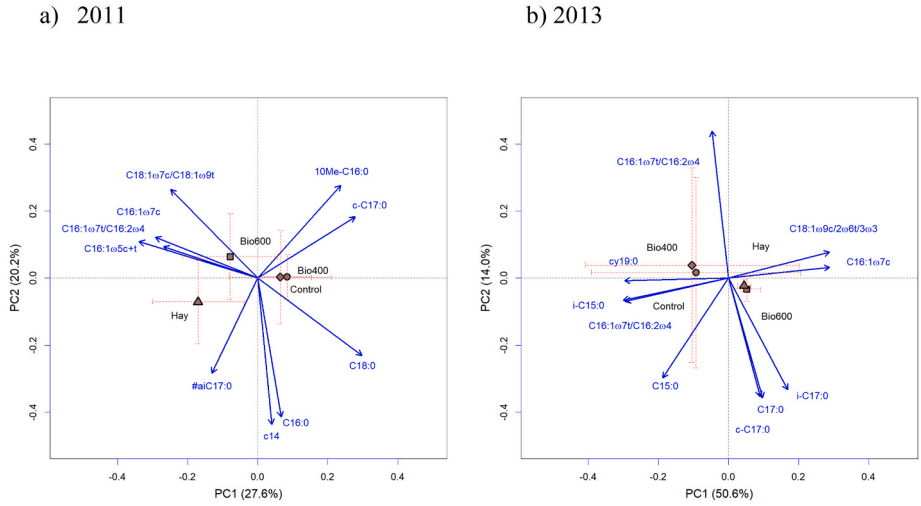

Fig. 6. Principal Component Analysis of 21 PLFAs (those with non-zero variance). The top ten eigenvectors (based on absolute value) for each PC axis are shown. The treatment mean eigenvalues are shown with error bars (±1 standard deviation in red). Analyses are shown separately for 2011 (a) and 2013 (b). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

composition and abundance (Leff et al., 2018). In 2011, the plant community composition in the biochar plots was strongly dominated by legumes. Our experiment lasted three years, covering three full vegetative cycles. The composition of the plant communities was strongly influenced by the type of amendment during the first growth season (Fig. 2, and also van de Voorde et al., 2014). As such H3 should be accepted. Plant productivity was likely affected by nutrients in the biochar ash fraction van de Voorde et al. (2014).

This increase in legume abundance in the first year was likely caused by increased K availability, as described by van de Voorde et al. (2014). But, in 2013, legumes no longer dominated. Therefore H5 should also be accepted plants – the impacts of the treatments reduced overtime. However, it is notable that the levels of available K in the soil were still much higher in the biochar amended plots than in Control and Hay plots. While biochar effects on the development of subsequent plant communities could have been extended, for example due to increased seed production with consequences for community composition in future seasons, in our experiment, vegetation developed a closed canopy rapidly after sowing such that habitat niches for germination during the second and third year became limited. Furthermore, at the end of the growth season, the plots were mown and the biomass was removed. This practice may have also removed many of the seeds before they could be distributed onto the plots. Mowing could also explain why the grass cover increased strongly in all treatments and became the dominant functional group as grasses typically do well when mown and regrow rapidly.

4.4. *Limitations of the study*

With the experimental design used it is not possible to elucidate the drivers or any observed differences. Feedback cycles between plant communities and the soil biota are well known (e.g. Wardle et al., 2004). Here, it is possible that the treatments impacted the plant communities, which then in turn affected the abundance and community composition of some of the soil organisms.

5. Implications

The addition of the biochar produced from meadow plant cuttings produced transient effects over the three-year timeframe of this study. The biochar effects were most pronounced in the plant community, where we observed an increase in legume cover. This suggests that this type of biochar may have benefits for plant productivity, especially for legumes, which could benefit the whole plant community when, for example, including legumes in plant species mixtures for cover crops or pasture planting. The addition of meadow plant cuttings, here referred to as Hay, stimulated soil biota but not when added in the form of biochar, yet the effects were largely transient over the 3-year timeframe of this experiment and were more apparent in soil microbes and microfauna (nematodes) than in other functional groups. While we acknowledge that our findings are site and biochar type specific, these results suggest that biochar used here, produced from the local plant community, can be applied to soil, at least at the 10 t/ha rate used in this experiment, with little to no detrimental effects on the soil biota. This suggests that biochar application to soil in restoration grasslands on former arable fields could be used as a climate change mitigation tool with the potential to increase soil C storage without negative impacts on the soil biota. However, as other studies have shown biochar to interact with soil organic matter through both positive and negative priming, further work is needed to investigate that interaction and elucidate mechanisms before biochar application can be recommended under different conditions from those used in this trial. Furthermore, as each type of biochar might interact differently with soil organisms in different ecosystems, more field studies including multitrophic investigations are required to confirm the generality of the results presented by this study. This is particularly important in the case of biochar, because once biochar is added it is not possible to remove it from the soil in a cost-effective manner.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108789.

References

Bardgett, R., 2005. The Biology of Soil: A Community and Ecosystem Approach. Oxford University Press.
Bedano, J.C., Cantú, M.P., Doucet, M.E., 2006. Influence of three different land management practices on soil mite (Arachnida: Acari) densities in relation to a natural soil. Applied Soil Ecology 32 (3), 293–304.
Blanco-Canqui, H., 2021. Does biochar improve all soil ecosystem services? GCB Bioenergy 13, 291–304. https://doi.org/10.1111/gcbb.12783.
Bongers, T., Bongers, M., 1998. Functional diversity of nematodes. Applied Soil Ecology 10 (3), 239–251.
Borcard, D., Gillet, F., Legendre, P., 2011. Numerical Ecology with R, *Use R!* Springer-Verlag, New York.
Briones, M.J.I., Panzacchi, P., Davies, C.A., Ineson, P., 2020. Contrasting responses of macro-and meso-fauna to biochar additions in a bioenergy cropping system. Soil Biology and Biochemistry 145, 107803.
Budai, A., Rasse, D.P., Lagomarsino, A., Lerch, T.Z., Paruch, L., 2016. Biochar persistence, priming and microbial responses to pyrolysis temperature series. Biology and Fertility of Soils 52 (6), 749–761.
Callaham Jr., M.A., Richter Jr., D.D., Coleman, D.C., Hofmockel, M., 2006. Long-term land-use effects on soil invertebrate communities in Southern Piedmont soils, USA. European Journal of Soil Biology 42, S150–S156.
Cambardella, C.A., Elliott, E.T., 1994. Carbon and nitrogen dynamics of soil organic matter fractions from cultivated grassland soils. Soil Science Society of America Journal 58 (1), 123–130.
Domene, X., Hanley, K., Enders, A., Lehmann, J., 2015. Short-term mesofauna responses to soil additions of corn stover biochar and the role of microbial biomass. Applied Soil Ecology 89, 10–17.
Enders, A., Hanley, K., Whitman, T., Joseph, S., Lehmann, J., 2012. Characterization of biochars to evaluate recalcitrance and agronomic performance. Bioresource Technology 114, 644–653.
Fidel, R.B., Laird, D.A., Thompson, M.L., Lawrinenko, M., 2017. Characterization and quantification of biochar alkalinity. Chemosphere 167, 367–373.
Frostegård, Å., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22 (1–2), 59–65.
Graefe, U., Schmelz, R.M., 1999. Indicator values, strategy types and life forms of terrestrial Enchytraeidae and other microannelids. Newsletter on Enchytraeidae 6, 59–67.
Gruss, I., Twardowski, J.P., Latawiec, A., Medyńska-Juraszek, A., Królczyk, J., 2019. Risk assessment of low-temperature biochar used as soil amendment on soil mesofauna. Environmental Science and Pollution Research 26 (18), 18230–18239.
Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., Deng, H., 2015. Physico-chemical properties and microbial responses in biochar-amended soils: mechanisms and future directions. Agriculture, Ecosystems & Environment 206, 46–59.
Gundale, M.J., DeLuca, T.H., 2006. Temperature and source material influence ecological attributes of ponderosa pine and Douglas-fir charcoal. Forest Ecology and Management 231 (1–3), 86–93.
Herath, H.M.S.K., Camps-Arbestain, M., Hedley, M., 2013. Effect of biochar on soil physical properties in two contrasting soils: an Alfisol and an Andisol. Geoderma 209, 188–197.
Ippolito, J.A., Cui, L., Kammann, C., Wrage-Mönnig, N., Estavillo, J.M., Fuertes-Mendizabal, T., Cayuela, M.L., Sigua, G., Novak, J., Spokas, K., Borchard, N., 2020. Feedstock choice, pyrolysis temperature and type influence biochar characteristics: a comprehensive meta-data analysis review. Biochar 2 (4), 421–438.
Jeffery, S., Bezemer, T.M., Cornelissen, G., Kuyper, T.W., Lehmann, J., Mommer, L., Sohi, S.P., van de Voorde, T.F., Wardle, D.A., van Groenigen, J.W., 2015. The way forward in biochar research: targeting trade-offs between the potential wins. GCB Bioenergy 7 (1), 1–13.
Jeffery, S., Abalos, D., Prodana, M., Bastos, A.C., Van Groenigen, J.W., Hungate, B.A., Verheijen, F., 2017. Biochar boosts tropical but not temperate crop yields. Environmental Research Letters 12 (5), 053001.
Kasozi, G.N., Zimmerman, A.R., Nkedi-Kizza, P., Gao, B., 2010. Catechol and humic acid sorption onto a range of laboratory-produced black carbons (biochars). Environmental Science and Technology 44 (16), 6189–6195.
Klamer, M., Bååth, E., 2004. Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18: 2ω6, 9. Soil Biology and Biochemistry 36 (1), 57–65.
Laybourn-Parry, J., 1984. Trophic relations of Protozoa. In: A Functional Biology of Free-Living Protozoa. Springer, Boston, MA, pp. 135–163.
Leff, J.W., Bardgett, R.D., Wilkinson, A., Jackson, B.G., Pritchard, W.J., Jonathan, R., Oakley, S., Mason, K.E., Ostle, N.J., Johnson, D., Baggs, E.M., 2018. Predicting the structure of soil communities from plant community taxonomy, phylogeny, and traits. The ISME Journal 12 (7), 1794–1805.
Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems–a review. Mitigation and Adaptation Strategies for Global Change 11 (2), 403–427.
Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota–a review. Soil Biology and Biochemistry 43 (9), 1812–1836.
Liu, X., Zhang, A., Ji, C., Joseph, S., Bian, R., Li, L., Pan, G., Paz-Ferreiro, J., 2013. Biochar's effect on crop productivity and the dependence on experimental conditions—a meta-analysis of literature data. Plant and Soil 373, 583–594. https://doi.org/10.1007/s11104-013-1806-x.
Llovet, A., Mattana, S., Chin-Pampillo, J., Gascó, G., Sánchez, S., Mondini, C., Briones, M. J.I., Márquez, L., Alcañiz, J.M., Ribas, A., Domene, X., 2021. Long-term Effects of Gasification Biochar Application on Soil Functions in a Mediterranean Agroecosystem: Higher Addition Rates Sequester More Carbon but Pose a Risk to Soil Faunal Communities, vol. 801. Science of The Total Environment, 149580.
Macfadyen, A., 1961. Improved funnel-type extractors for soil arthropods. Journal of Animal Ecology 171–184.
Marks, E.A., Mattana, S., Alcañiz, J.M., Domene, X., 2014. Biochars provoke diverse soil mesofauna reproductive responses in laboratory bioassays. European Journal of Soil Biology 60, 104–111.
McCormack, S.A., Ostle, N., Bardgett, R.D., Hopkins, D.W., Vanbergen, A.J., 2013. Biochar in bioenergy cropping systems: impacts on soil faunal communities and linked ecosystem processes. GCB Bioenergy 5 (2), 81–95.

Mukherjee, A., Lal, R., 2013. Biochar impacts on soil physical properties and greenhouse gas emissions. Agronomy 3 (2), 313–339.
Munksgaard, N.C., McBeath, A.V., Ascough, P.L., Levchenko, V.A., Williams, A., Bird, M. I., 2019. Partitioning of microbially respired CO 2 between indigenous and exogenous carbon sources during biochar degradation using radiocarbon and stable carbon isotopes. Radiocarbon 61 (2), 573–586.
Nielson, U.N., Ayres, E., Wall, D.H., Bardgett, R.D., 2011. Soil biodiversity and carbon cycling: a review and synthesis of studies examining diversity–function relationships. European Journal of Soil Science 62 (1), 105–116.
O'connor, F.B., 1955. Extraction of enchytraeid worms from a coniferous forest soil. Nature 175 (4462), 815–816.
Oostenbrink, M., 1960. Estimating Nematode Populations by Some Selected Methods. Nematology, pp. 85–102.
Oram, N.J., van de Voorde, T.F., Ouwehand, G.J., Bezemer, T.M., Mommer, L., Jeffery, S., Van Groenigen, J.W., 2014. Soil amendment with biochar increases the competitive ability of legumes via increased potassium availability. Agriculture, Ecosystems & Environment 191, 92–98.
Quilliam, R.S., Glanville, H.C., Wade, S.C., Jones, D.L., 2013. Life in the 'charosphere' – does biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biology and Biochemistry 65, 287–293. https://doi.org/10.1016/j.soilbio.2013.06.004.
R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.
Rabbi, S.M.F., Daniel, H., Lockwood, P.V., Macdonald, C., Pereg, L., Tighe, M., Wilson, B. R., Young, I.M., 2016. Physical soil architectural traits are functionally linked to carbon decomposition and bacterial diversity. Scientific Rep. 6 (1), 1–9.
Reibe, K., Götz, K.P., Roß, C.L., Döring, T.F., Ellmer, F., Ruess, L., 2015. Impact of quality and quantity of biochar and hydrochar on soil Collembola and growth of spring wheat. Soil Biology and Biochemistry 83, 84–87.
Rønn, R., Thomsen, I.K., Jensen, B., 1995. Naked amoebae, flagellates, and nematodes in soils of different texture. European Journal of Soil Biology 31 (3), 135–141.
Schnee, L.S., Knauth, S., Hapca, S., Otten, W., Eickhorst, T., 2016. Analysis of physical pore space characteristics of two pyrolytic biochars and potential as microhabitat. Plant and Soil 408 (1–2), 357–368.
Spokas, K.A., 2010. Review of the stability of biochar in soils: predictability of O:C molar ratios. Carbon Management 1 (2), 289–303.
Tammeorg, P., Bastos, A.C., Jeffery, S., Rees, F., Kern, J., Graber, E.R., Ventura, M., Kibblewhite, M., Amaro, A., Budai, A., Cordovil, C.M.D.S., 2017. Biochars in soils: towards the required level of scientific understanding. Journal of Environmental Engineering and Landscape Management 25 (2), 192–207.
van de Voorde, T.F., Bezemer, T.M., Van Groenigen, J.W., Jeffery, S., Mommer, L., 2014. Soil biochar amendment in a nature restoration area: effects on plant productivity and community composition. Ecological Applications 24 (5), 1167–1177.
Wang, J., Xia, K., Waigi, M.G., Gao, Y., Odinga, E.S., Ling, W., Liu, J., 2018. Application of biochar to soils may result in plant contamination and human cancer risk due to exposure of polycyclic aromatic hydrocarbons. Environment International 121, 169–177.
Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., Van Der Putten, W.H., Wall, D. H., 2004. Ecological linkages between aboveground and belowground biota. Science 304 (5677), 1629–1633.
Xu, N., Tan, G., Wang, H., Gai, X., 2016. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. European Journal of Soil Biology 74, 1–8.
Ye, L., Camps-Arbestain, M., Shen, Q., Lehmann, J., Singh, B., Sabir, M., 2020. Biochar effects on crop yields with and without fertilizer: a meta-analysis of field studies using separate controls. Soil Use & Management 36 (1), 2–18.
Yeates, G.W., Bongers, T.D., De Goede, R.G.M., Freckman, D.W., Georgieva, S.S., 1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. Journal of Nematology 25 (3), 315.
Zhao, L., Cao, X., Mašek, O., Zimmerman, A., 2013. Heterogeneity of biochar properties as a function of feedstock sources and production temperatures. Journal of Hazardous Materials 256, 1–9.
Zheng, H., Liu, W., Zheng, J., Luo, Y., Li, R., Wang, H., Qi, H., 2018. Effect of long-term tillage on soil aggregates and aggregate-associated carbon in black soil of Northeast China. PLoS One 13 (6), e0199523.
Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. Mixed Effects Models and Extensions in Ecology with R, *Statistics For Biology And Health*. Springer-Verlag, New York.