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Biochar and peat amendments affect nitrogen retention, microbial capacity and nitrogen cycling microbial communities in a metal and polycyclic aromatic hydrocarbon contaminated urban soil

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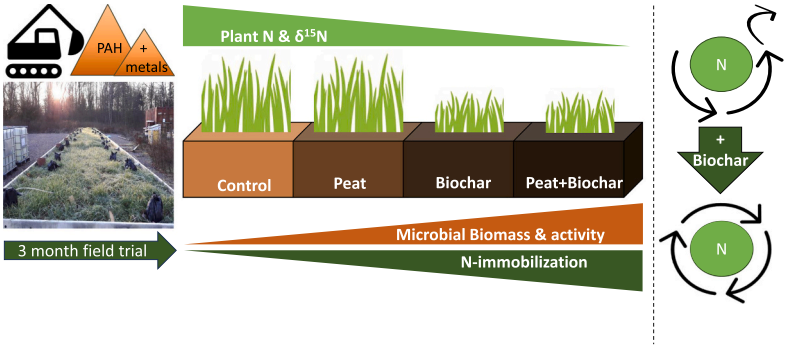
HIGHLIGHTS

- Three-month outdoor field trial with a metal and PAH co-contaminated urban soil
- Microbial guilds *nrfA*, *nirK*, *nosZI* and *nosZII* increased with biochar and peat
- Lower $\delta^{15}N$ suggesting lower gaseous N losses with biochar and peat
- Biochar and peat amendment differentially affected microbial substrate utilization
- Biotic nitrogen immobilization through growing microbial biomass

GRAPHICAL ABSTRACT



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ABSTRACT

Soil contaminants may restrict soil functions. A promising soil remediation method is amendment with biochar, which has the potential to both adsorb contaminants and improve soil health. However, effects of biochar amendment on soil-plant nitrogen (N) dynamics and N cycling microbial guilds in contaminated soils are still poorly understood. Here, a metal- and polycyclic aromatic hydrocarbon (PAH) contaminated soil was amended with either biochar (0, 3, 6 % w/w) and/or peat (0, 1.5, 3 % w/w) in a full-factorial design and sown with perennial ryegrass in an outdoor field trial. After three months, N and the stable isotopic ratio $\delta^{15}N$ was measured in soil, roots and leaves, along with microbial responses. Aboveground grass biomass decreased by 30 % and leaf N content by 20 % with biochar, while peat alone had no effect. Peat in particular, but also biochar, stimulated the abundance of microorganisms (measured as 16S rRNA gene copy number) and basal respiration. Microbial substrate utilization (MicroResp™) was altered differentially, as peat increased respiration of all carbon sources, while for biochar, respiration of carboxylic acids increased, sugars decreased, and was unaffected for amino

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acids. Biochar increased the abundance of ammonia oxidizing archaea, while peat stimulated ammonia oxidizing bacteria, *Nitrobacter*-type nitrite oxidizers and comB-type complete ammonia oxidizers. Biochar and peat also increased nitrous oxide reducing communities (*nosZI* and *nosZII*), while peat alone or combined with biochar also increased abundance of *nirK*-type denitrifiers. However, biochar and peat lowered leaf $\delta^{15}N$ by 2–4 ‰, indicating that processes causing gaseous N losses, like denitrification and ammonia volatilization, were reduced compared to the untreated contaminated soil, probably an effect of biotic N immobilization.

Overall, this study shows that in addition to contaminant stabilization, amendment with biochar and peat can increase N retention while improving microbial capacity to perform important soil functions.

1. Introduction

Soil contamination is widespread and is caused by the release of chemicals from a diverse range of sources associated with fossil fuel burning, manufacturing, agriculture, infrastructure and technology (European Environment Agency, 2020). The most common soil contaminants in historically contaminated sites include heavy metals (35 %), mineral oils (24 %) and polycyclic aromatic hydrocarbons (PAH; 11 %) (Panagos et al., 2013). Soil contamination affects soil organisms, plant growth and higher wildlife, thereby decreasing soil functionality and biodiversity with knock-on effects for human health (FAO and UNEP, 2021). Soil nitrogen (N) cycling in particular has been shown to be sensitive to long-term contaminant exposure (Jacquiod et al., 2018; Lu et al., 2022; Rijk and Ekblad, 2020), either due to direct toxicity but more often because of indirect changes in the soil environment such as substrate availability (Fleeger, 2020; Rijk et al., 2023). When contaminated soils are remediated, focus should not only be on contaminant removal, destruction or immobilization, but also on the restoration of impaired ecological soil functions in order to regain soil fertility and restore healthy nutrient cycling (Banning et al., 2008; Epelde et al., 2014). In the light of the upcoming EU Soil Monitoring Law as part of the EU Soil Strategy, restoration efforts of degraded and/or contaminated areas will be increasingly required to achieve the objective of healthy soils by 2050 (European Commission, 2024). Therefore, mechanistic understanding on ecological effects of soil remediation methods to restore soil health is urgently needed.

A potential remediation strategy is the use of organic soil amendments which increases the circular use of moderately contaminated soils by immobilizing pollutants and increasing soil quality (Kumpiene et al., 2019; Palansooriya et al., 2020). This approach can bring large reductions of CO_2-emissions compared to landfilling (Papageorgiou et al., 2021).

Among soil amendments, biochar application is seen as one of the most promising environmentally friendly and cost-efficient methods for remediation of contaminated soils (Beesley et al., 2011; Oliveira et al., 2017). Biochar is a carbon-rich substance produced by pyrolysis of biological residues and can be an effective long-term sorbent for diverse soil contaminants, thus disrupting potential 'source-pathway-receptor' linkages (Beesley et al., 2011; Sizmur et al., 2016). Biochar also holds potential co-benefits, such as promoting microbial activity, biomass and diversity, plant growth and nutrient sequestration (Biederman and Harpole, 2013; Gao et al., 2019; Li et al., 2020; Spokas et al., 2012). Amendment with biochar has been shown to alter processes involved in biogeochemical cycling of key nutrients in soils, in particular N (Gul and Whalen, 2016). This is due to both direct effects, whereby biochar provides a niche for opportunistic microorganisms, and indirect effects, such as the provision of labile substrates from fresh biochar, alteration of soil pH and moisture, and sorption of signal molecules (Gul et al., 2015). In addition to biochar, other organic amendments such as peat may offer an opportunity to immobilize pollutants (Liu et al., 2022; Rydin et al., 2013) as well as to increase organic matter content, moisture retention and aeration (Ekwue and Harrilal, 2010; Vestberg et al., 2009). While peat is expected to degrade more rapidly and have a weaker immobilization effect on organic soil pollutants than biochar, it may still stimulate the microbial community, assisting in the recovery of soil health (Niemi et al., 2008; Vepsäläinen et al., 2004).

The effect of biochar and peat amendments on soil N cycling in contaminated soil where N cycling organisms may be suppressed, is yet not fully understood. Short-term laboratory studies applying biochar to either field- or artificially contaminated soils showed enhanced nitrification and denitrification activity and/or changes in the abundance of N cycling communities, but results are context-dependent and especially sensitive to soil nutrient levels (Chen et al., 2017; Li et al., 2019; Li et al., 2021; Liu et al., 2020; Su et al., 2019; Zhang et al., 2017; Zhao et al., 2020). So far, only a few studies have explored alterations to microbial communities and their activities in historically contaminated urban soils with vegetation and under outdoor conditions in Nordic climates (Cui et al., 2023; Mackie et al., 2015; Zhou et al., 2018).

To follow overall changes of biochar and other amendments to the soil N cycle, stable isotope signatures of nitrogen, $\delta^{15}N$, may be used (Craswell et al., 2021) as $\delta^{15}N$ provides an integrative measure of various biotic and abiotic N processes (Craine et al., 2015; Dawson et al., 2002; Högberg, 1997). When N is transformed or lost from the soil, microbial and abiotic processes preferentially 'use' the lighter isotope ^{14}N, thereby enriching the remaining soil N pool in ^{15}N (Högberg, 1997; Högberg and Johannisson, 1993). Gaseous N losses are thought to be primarily responsible for large-scale plant and soil $\delta^{15}N$ patterns (Houlton and Bai, 2009), with high fractionation occurring with denitrification of NO_2^- to NO (-19.8 ± 7.6 ‰) and NH_3 volatilization (-40 to -60 ‰) (Denk et al., 2017). Losses of N by leaching are not associated with large isotopic fractionation as suggested by studies across various terrestrial ecosystems showing total leached N to have similar $\delta^{15}N$ values as soil organic matter (Mnich and Houlton, 2016). To examine effects on the potential for specific transformations, quantification of microbial groups that drive various N-cycle processes can be done using marker genes that encode key enzymes in different pathways, e.g. N-fixation (*nifH*), nitrification (*amoA*, *nxr*) and denitrification pathways (*nirK*, *nirS*, *nosZ*) (Levy-Booth et al., 2014; Schloter et al., 2018). Previous work has shown that changes in abundances of the different groups often reflect differences in N availability or other environmental conditions in soils (Enwall et al., 2010; Jones and Hallin, 2019; Prosser and Nicol, 2012). To provide better insight into time- and process integrated changes to the soil N cycle and microbially-mediated reactions thereof in the soil-plant system, we therefore propose a combined approach of measurements of natural abundance of ^{15}N and measurements of the abundance of N cycling microbial communities (Bai et al., 2015).

Based on the above, we established an outdoor field trial in southern Sweden to develop a sustainable biochar-based remediation technique for PAH- and metal contaminated soils using biochar and peat amendments (Enell et al., 2020) with the aim to restore soil health and facilitate the re-use of soil. The objective of the present paper is to examine the effects of separate and combined amendments on soil N cycling during the first growing season, including effects on microorganisms, plants and soil physicochemical properties. We hypothesized that both biochar and peat would increase microbial biomass and respiration as well as the abundance of N cycling microbial communities in the amended soil. However, differences in microbial and plant responses are expected between amendments with biochar and peat, as biochar will likely act as a stronger sorbent while peat supplies more nutrients,

microorganisms and contains more mineralizable carbon (C). The response of the different N transforming microbial communities will therefore reflect their niche preference (e.g. available substrates, soil physicochemical properties) as well as potential sensitivity to soil contaminants.

2. Materials and methods

2.1. Soil, biochar and peat

Contaminated soils from two different sites in Sweden were combined into a single 'control' soil prior to biochar and peat amendment. One of the two soils was a metal-contaminated soil (approximate concentrations: lead (Pb) 1000 mg kg^{-1} dw, copper (Cu) 400 mg kg^{-1} dw and zinc (Zn) 600 mg kg^{-1} dw) which originated from excavation works at 1–2 m depth of a port area in Helsingborg, southern Sweden. This soil was dominated by sand and silt, and contained fragments of glass, ceramics, and metal. The other soil was contaminated with PAH and originated from a gasworks site in Helsingborg (400 mg kg^{-1} dw Σ16-EPA PAH). This soil was sampled between 0.4 and 1 m depth and contained stones and wastes in the form of slags and charred materials. To achieve a homogeneous distribution of the contaminants, each soil was sieved using a rotating cylinder (40 mm) and the two soils were mechanically mixed in a ratio of 80:20 ('metal' soil:'PAH' soil; w/w) using an excavator that lifted and dropped soil portions at different locations at the pile for over 50 times. Sieved garden compost (15 mm) was added at 1 % (w/w) as an inoculum and organic fertilizer, before a second sieving (40 mm) and full homogenization using the same techniques. This homogenized 'control soil' (4 % clay, 6 % silt, 69 % sand, 21 % gravel of 2–4 mm) was then divided into nine piles of about 9000 kg soil each. The total metal and PAH content of the control soil is provided in Table 1.

The biochar was selected based on a previous study examining several types of biochar for sorption capacity for both organic and inorganic contaminants, as well as their recalcitrance in soil (Enell et al., 2020). The selected biochar (IKB Innsbruck, Austria) was produced out of wood chips and bark using pyrolysis with a floating bed reactor at 750 °C for 20 min. The biochar had a pH_{CaCl2} of 8.6, along with a specific surface area of >205 m^2 g^{-1}, a total organic carbon (TOC) content of 86.1 %, an oxygen/carbon (O/C) ratio of 0.036, and an ash content of 9.8 %. Total content of N was 0.5 % and phosphorus (P) content 0.1 %. Results of a full European Biochar Certificate (EBC) analysis are shown in Supplemental Table S1. The peat (Hasselfors Garden, Sweden) had a humification degree of 4–5 on the von-Post scale and an initial pH of 4.4, which was limed with 1.7 % w/w Cresco Vital limestone (SMA Minerals, Filipstad) to increase the pH to 5.9. The peat was sieved (15 mm) before and after liming.

Details on the total carbon (TC), TOC, $\delta^{13}C$, N and $\delta^{15}N$ content of the original soils, peat, biochar, compost and fertilizer (see Section 2.2) are given in Supplemental Table S2.

Table 1
Summary of metal and PAH content of the control soil used in the field trial; means (± sd); n = 3; in mg kg^{-1} dw.

Contaminant	Content (mg kg^{-1} dw)
As	8.3 (0.36)
Ba	297 (15.8)
Pb	473 (123)
Cd	0.4 (0.11)
Co	5.2 (0.17)
Cu	237 (63.5)
Cr	16.3 (1.15)
Hg	1.7 (0.06)
Mo	< 2.1
Ni	13.3 (0.58)
Sb	8.7 (3.06)
V	21.3 (0.58)
Zn	417 (60.3)
Σ16PAH (US-EPA)	81.1 (12.1)

2.2. Treatments and experimental design

The field trial was established in June 2019 at the premises of the municipal waste management facility 'NSR', Helsingborg, Sweden. The control soil was mixed with 0, 2.8 or 5.6 % (w/w) biochar (BC) and 0, 1.5 or 2.9 % (w/w) peat (P) in a 3 × 3 full-factorial design. The amount of biochar was selected based on a literature review, reporting positive effects up to 5 % addition of biochar (Bielská et al., 2018; Khan et al., 2015; Oleszczuk et al., 2014), but negative effects on the soil ecosystem at 10 % (Bielská et al., 2018). While we intended to add a similar amount of peat, the actual dry weight of the outdoor stored peat was considerably different from its initial conditions, resulting in a lower w/w addition compared to biochar. Aiming for thorough homogenization, the peat and biochar were mixed into the soil mechanically to thoroughly homogenize the soil, again using an excavator and passing it through a rotating cylinder with holes of 40 mm. The control soil without peat and biochar amendments, (BC0P0), was treated in the same way. Resulting mixtures were divided into three replicates. Each replicate was randomly placed in a 2 × 2 m cultivation bed in one of three rows, with a distance between rows of six meters. Beds had a soil depth of 0.4–0.5 m on top of a permeable geotextile (1.5 mm) which separated the soil from a 0.2 m drainage layer of macadam (size fraction of 8–11 mm) which was placed on a 1 mm thick impermeable geotextile (EPDM; DuPont™).

Perennial ryegrass (*Lolium perenne* L.; variety Indicus 1, Olssons Frö AB, Helsingborg) was sown (3 g m^{-2}) on June 28, 2019. Before sowing, cultivation beds were watered (25 mm on 27 June 2019 and 25 mm on 28 June 2019) and 19 g m^{-2} fertilizer containing 20.6 % N (12.7 % ammonium, NH_4^+, and 7.9 % nitrate, NO_3^-), 3.6 % P, 6.6 % K, 0.9 % Mg and 3 % S (YaraMila) was mixed into the surface soil. Ryegrass was selected as it is commonly used as turf grass and in pastures in Sweden and responds well to fertilization treatments, making it a useful cover crop for soil stabilization (Popay, 2022). During the experiment, precipitation was recorded, and the cultivation beds were watered up to 20 mm/week if the weekly precipitation was <20 mm. In Mid-August all plots were cleared from weeds, mostly tomato plants, which competed with the ryegrass in all plots. On August 28, additional ryegrass was sown (1.5 g m^{-2}) where grass was sparse. Any upcoming weed was thereafter continuously removed from the beds.

2.3. Sampling of soil and grass

Soil and grass were sampled September 25–27, 2019. Each cultivation bed was divided into nine equal squares. Above ground grass biomass was collected from all but the central square of each bed by cutting all leaves and stalks within an area of 0.04 m^2 in each square marked by a 20 × 20 cm wooden frame, making up a total sampled area of 0.32 m^2 of each cultivation bed. One or two soil cores (diam. 7 cm; 20 cm depth) were collected from each grass sampling area, collecting 10–16 cores in total from each cultivation bed. Soil samples were sieved (<2 mm) and homogenized in the field and combined into one composite sample to account for spatial variability within each replicate bed, before splitting into subsamples of soil and roots for different analyzes. In October and December 2019, the soil solution was sampled from a soil depth of 25 cm using lysimeters (Prenart Super Quartz, PTFE, DMR Equipment, Denmark).

Subsamples of grass leaves (living tissue only) were washed with MilliQ water. Roots were cleaned by soaking for 20 min in cold tap water and subsequently rinsing with cold tap water in repeated cycles to clean out the soil, peat and biochar. Remaining soil, peat- and biochar particles were cleared using tweezers by visual inspection under magnifiers. Subsamples of cleaned grass leaves, roots, soil from the treatments and the primary materials (peat, biochar, original soils etc.) were freeze-

dried. Leaves and roots were cut into small pieces and homogenized.

2.4. Chemical analysis

Soil pH in water was determined according to SS-ISO 10390, exchangeable cations (CEC; Na, Ca, K and Mg) were determined with inductively coupled plasma sector field mass spectroscopy (ICP- SFMS) after extraction with 1 M NH_4Cl (Swedish Standard SS 02 83 13). The content of available P in soil was analyzed by Olsen P (Olsen and Sommers, 1965). Soil elemental contents of As, Ba, Cd, Co, Cr, Cu, Hg, Ni, Pb, V and Zn were determined with inductively coupled plasma atomic emission spectroscopy (ICP-AES) after digestion with 7 M HNO_3; Mo and Sb were determined with ICP-MS after digestion with *aqua regia* (ISO17294-2). Extraction and analysis of PAH were performed according to Titaley et al. (Titaley et al., 2020). Total contents of metals and PAH of biochar- and peat treated soil are reported in Supplemental Table S3; high variability of Pb and Sb likely reflect the heterogeneous nature of the original metal contaminated soil. The soil solution samples were analyzed for pH, dissolved organic carbon (DOC) by combustion and infrared detection after acidification and removal of inorganic carbon, and ammonium, nitrate and phosphate were spectrophotometrically determined after filtration. Details on the methods and results of trace elements in the soil solution and grass leaves, as well as freely dissolved PAH porewater concentration (PAH-$C_{W,free}$) in the soil samples using the passive sampler polyoxymethylene (POM) (Arp et al., 2014; Enell et al., 2016) are reported elsewhere (Enell et al., 2020). To get an indication of the level of toxicity that is associated with the metal contents in the control soil and peat amended soil, soil-specific bioavailability corrected toxicity threshold values were calculated (ARCHE, 2020a; ARCHE, 2020b) (Supplemental Methods S1).

2.5. C and N elemental and stable isotopic measurements

Subsamples of freeze-dried soil, grass leaves, and roots were milled to powder with a ball-mill. An amount of 5 to 20 mg of soil, 2.5 mg of grass leaves and 3.0 mg of grass roots were weighed into tin capsules to measure the C and N content and isotopic signatures $\delta^{13}C$ and $\delta^{15}N$ with an elemental analyzer (vario PYRO cube EA; Elementar, Manchester, UK) coupled online to a continuous flow Isoprime precisION isotope ratio mass spectrometer (Elementar, Manchester, UK). For TOC and $\delta^{13}C$ analyses, soil samples were weighed into silver capsules and treated with hydrochloric acid (2 M HCl in 10 μl doses) to remove any possible carbonates. Acidified samples were oven-dried at 60 °C for 24 h. The results of the isotopic analyses were expressed as parts per thousand (‰) deviations, in the ratio of the heavy to the light isotope of each element, from the international standards (Vienna Pee Dee Belemnite, V-PDB, for ^{13}C, atmospheric N_2 for ^{15}N). For $\delta^{13}C$ and $\delta^{15}N$ the working standard used was a wheat flour with a $\delta^{13}C$ of −26.231 ‰ and a $\delta^{15}N$ of 5.817 ‰, calibrated with the international standards IAEA-CH6 for ^{13}C and IAEA-N1 for ^{15}N. The precision of analyses of 10 standard samples was better than 0.104 ‰ for $\delta^{15}N$ and 0.022 ‰ for $\delta^{13}C$.

2.6. Abundance of total and nitrogen cycling microbial communities

DNA was extracted in duplicate from approximately 0.5 g of soil (fresh weight) using the NucleoSPIN DNA extraction kit for soil (Macherry-Nagel, Germany) following the manufacturer's instructions. Final extracts were pooled, and the quality of DNA was examined by spectrophotometry as well as gel electrophoresis, followed by quantification using a Qubit fluorimeter (Thermo Scientific, Uppsala, Sweden).

Abundances of total bacterial communities, a proxy for microbial biomass, as well as abundances of various microbial functional groups involved in inorganic N transformation processes were determined by real-time quantitative PCR (qPCR) of 16S rRNA and specific marker genes encoding enzymes involved in each process (Supplemental Table S4). Prior to quantification, tests of PCR inhibition by co-extracted contaminants were performed for all samples by adding a known amount of pGEM-T plasmid (Promega Corporation, Madison WI, USA) to 5 ng of extracted soil DNA or controls containing 5 ng of non-target λ-phage DNA (Thermo Scientific, Uppsala, Sweden), followed by qPCR using plasmid-specific primers T7 and SP6 in 15 μl reactions. No inhibition of the PCR reactions was detected based on comparison of cycle threshold (Ct) values between DNA extracts and controls. Primer combinations and thermal cycling conditions used to quantify all genes are described in Supplemental Table S3. All reactions were performed on a CFX-connect Real-time PCR system (Bio-Rad, Hercules CA, USA) and contained iQ™ SYBR Green Supermix (Bio-Rad), 0.1 % bovine serum albumin (BSA; New England Biolabs, Ipswich MA, USA) and 5 ng DNA. Final primer concentrations varied between 0.5 μM for 16S rRNA, AOA- and AOB-*amoA* (ammonia oxidizing archaea and bacteria), *nxrB* (*Nitrospira* and *Nitrobacter* nitrite oxidizing bacteria), *nifH* (N fixation), *nrfA* (nitrate ammonification), *nosZ* clade I (nitrous oxide reduction) and *nirS* and *nirK* (denitrification), and 2.0 μM for comammox-*amoA* clades A and —B (comA, comB, complete nitrification) and *nosZ* clade II genes (nitrous oxide reduction). Each gene was quantified in duplicate runs of 15 μl reactions and the qPCR efficiencies ranged from 75.2 to 96.8 % ($R^2 > 0.99$ for all runs). All PCR products were inspected by melt-curve analysis as well as gel electrophoresis to ensure the absence of non-specific amplification products.

2.7. Basal respiration and microbial physiological response

The physiological response of the soil microbial communities was determined at the James Hutton Institute, UK, using the MicroResp™ soil respiration system (Campbell et al., 2003), on three technical replicates for each soil sample. Soil samples were stored at 4 °C during 2 months prior to determination, then preincubated at 25 °C for 3–5 days after arrival to the laboratory and 3–5 days after filling the deepwell plates (approx. 0.34 g dw soil per well). Solutions of sole C sources (L-alanine, α-cyclodextrin, α-ketoglutaric acid, L-arabinose, ascorbic acid, L-cysteine HCl, D-glucose, D-fructose, γ-amino butyric acid, L-glutamine, L-malic acid, *N*-acetyl-glucosamine, oxalic acid, protocatechuic acid, trehalose) or deionized water were then added and the carbon dioxide (CO_2) evolved over six hours of incubation at 25 °C was trapped in agar-sodium bicarbonate gels stained with cresol red. The trapped CO_2 was determined from the change in absorbance at 570 nm between the start and end of the incubation using a Vmax microplate reader (Molecular Devices, USA). The substrate-induced respiration was calculated as the difference in respiration after addition of C sources and that after addition of deionized water (Campbell et al., 2003).

2.8. Data analyses

Statistical analyses were performed in R 4.1.2. (R Core Team, 2022). Differences in response variables across peat and biochar treatments were assessed using two-way analysis of variance (ANOVA). Linear models were fit using the '*stats*' package in R. To ensure normality and homoscedasticity, the Shapiro-Wilk test and Levene's test were performed, and residuals were inspected for each model. Responses were transformed where necessary, and where applicable, post-hoc pair-wise comparisons of treatment means were performed using Tukey's honestly significant differences (HSD) tests. To obtain an overview of the data, principal components analysis (PCA) of plant and microbial responses of microbial functional guilds, total abundance and basal respiration was performed using the '*stats*' package and plotted using the '*ggbiplot*' package (Vu, 2011). Correlations between soil, plant and microbial responses were tested using the '*corrplot*' package version 0.9.2 (Wei and Simko, 2021) using Spearmans' rank correlations. Bar- and scatterplots were constructed using the '*ggplot2*' package version 3.3.5 (Wickham, 2016). Results were considered significant at a *P*-value of ≤ 0.05.

The substrate-induced respiration data were subjected to principal coordinate analysis (PCO) and distance-based redundancy analysis

(dbRDA) in Primer 6.1.13/Permanova+1.0.3 (Primer-E Ltd., Auckland, New Zealand) to elucidate the contribution of the individual C sources to the separation of samples and which soil environment variables that most likely had driven the physiological responses. After standardisation of respiration and normalisation of environmental data, the DistLM procedure was used applying Euclidean distances, BIC as selection criterion and a step-wise procedure. The variables included in the analysis were the soils organic carbon (SOC), C/N, CEC, and water-holding capacity (approximated as 100 % - dry matter content at the time of sampling), the grass biomass, and the pH, soluble organic C and concentrations of plant nutrients and inorganic and organic contaminants derived from leaching tests (Enell et al., 2020).

3. Results

3.1. Soil and soil solution properties

Amendment of soil with the highest levels of biochar and peat increased soil TOC by a factor of four; from 2.2 % in the control (BC0P0) to 8.0 % in BC6P3 (Table 2), whereas addition of 3 % of either biochar or peat resulted in a doubling of TOC at the end of the first growing season. Peat addition increased soil total N content slightly from 0.10 % in the control soil to 0.12 % in the treatment with 3 % peat, while the N-poor biochar did not affect the total N content detectably (Table 2). Biochar additions resulted in significantly increased C/N ratios, ranging from 23 in the control to 60–70 in treatments with 6 % biochar. Peat added at 3 % also increased the soil C/N ratio by 58 %, but when added in combination with biochar there was no additional effect of peat on the C/N ratio. The soil CEC increased with the addition of peat from 9 $cmol_c\ kg^{-1}$ in the control soil to 12 $cmol_c\ kg^{-1}$ in treatments with 3 % peat, but biochar had no effect on the soil CEC. The pH was mildly alkaline in the untreated soil (7.7), and no significant changes in the soil pH were observed except for the combined treatments of 3 % biochar with 3 % peat (pH 8.3), and 6 % biochar with 1.5 % peat (pH 8.5). The DOC in the soil solution was reduced five- to tenfold with the addition of biochar, but there was no effect of peat (Table 2). Peat and biochar did not significantly affect pH of the soil solution water. However, the phosphate concentration increased by 25–75 % with the addition of biochar and was significantly increased in the treatment with 6 % biochar and no or 3 % added peat, compared to unamended soil. The concentration of NH_4^+ and NO_3^- in the soil solution was negligible for all treatments (Table 2).

3.2. Overall effect of biochar and peat on plant and microbial properties

Principal component analysis showed that biochar amendment was the main driver of overall differences in plant properties across all samples, with samples from different biochar treatments segregating along the first principal component (PC1) explaining 56.2 % of the variation (Fig. 1a). Samples with different peat amendment levels also segregated along PC1, however this trend was limited to within each biochar amendment level, indicating a weaker contribution of peat to differences in plant properties compared to biochar. Leaf and root N and $\delta^{15}N$ as well as leaf and root $\delta^{13}C$ were overall higher in soil without biochar with significant positive correlations across these variables (Supplemental Fig. S1). Differences in root and leaf N properties were reflected in the increased C/N ratios observed in peat and biochar amended soil, whereas no clear association was observed with differences in root biomass.

Peat had a strong effect on microbial properties, as samples largely clustered according to peat amendment along PC1, explaining 40.3 % of overall variation in microbial properties (Fig. 1b). Several properties that contributed strongly to variation in PC1 (microbial biomass, *nrfA*, *nirK*, *nosZ* I and II abundances) increased with either biochar or peat amendment but did not increase further when added together (Section 3.5) and correlated positively to soil TOC and CEC (Supplemental Fig. S2b). Also, biochar had a significant effect on microbial properties, although the effect was less pronounced compared to peat. Samples with different biochar amendments generally segregated along PC2 explaining 22.6 % of the total variation in microbial properties, however this pattern was not consistent across different peat amendments. This was associated with higher AOA, nitrite oxidizing bacteria *Nitrospira* (NIS), *nifH* and Comammox clade A abundances in soil with biochar only. Abundances of these groups also were negatively correlated with soil TOC, soil pH and/or soil CEC (Supplemental Fig. S2b).

3.3. Plant biomass, N and $\delta^{15}N$

Biochar amendments strongly affected aboveground grass biomass which decreased by 30 to 40 % from around 400 g m^{-2} to between 240

Table 2
Soil and soil solution properties (mean ± sd, n = 3) in a metal- and PAH contaminated soil treated with biochar (BC; in % of dry matter) and peat (P; in % of dry matter) in an outdoor field trial. Letters indicate significant differences (Tukey's honestly significant differences test, $P < 0.05$) between means for each soil property. Significant differences compared to the unamended control soil for each property are indicated in bold. Results of two-way ANOVA tests are provided in Supplemental Table S5.

Treatment	Soil							Soil solution				
	pH	TC (%)	TOC (%)	N (%)	C/N	eCEC ($cmol_c\ kg^{-1}$)	P-Olsen (mg 100 g^{-1})	pH	DOC (mg L^{-1})	NO_3^- (mg L^{-1})	NH_4^+ (mg L^{-1})	PO_4^{3-} (mg L^{-1})
BC0P0	7.7 (0.24)^{ab}	3.9 (0.11)^{a}	2.2 (0.10)^{a}	0.10 (0.001)^{a}	23.2 (0.95)^{a}	9.0 (0.35)^{a}	2.3 (0.10)^{a}	7.8 (0.13)^{a}	26 (2.8)^{d}	0.30 (0.11)	<0.050	0.16 (0.03)^{a}
BC3P0	7.7 (0.06)^{ab}	**6.1 (0.77)^{bc}**	**4.7 (0.96)^{c}**	0.10 (0.003)^{a}	**48.6 (10.9)^{cd}**	9.6 (0.59)^{ab}	2.4 (0.06)^{a}	8.0 (0.13)^{a}	**3 (0.3)^{ab}**	0.31 (0.14)	<0.050	0.23 (0.03)^{ab}
BC6P0	7.7 (0.07)^{ab}	**7.7 (0.44)^{d}**	**6.8 (0.83)^{de}**	0.10 (0.005)^{ab}	**68.3 (5.01)^{f}**	9.7 (0.57)^{abc}	2.5 (0.00)^{a}	7.9 (0.08)^{a}	**2 (0.5)^{a}**	<0.27	<0.050	**0.28 (0.02)^{b}**
BC0P1.5	7.7 (0.17)^{ab}	4.3 (0.49)^{a}	3.4 (0.56)^{ab}	0.11 (0.010)^{abc}	31.8 (7.69)^{ab}	**10.4 (0.56)^{bc}**	2.4 (0.10)^{a}	7.8 (0.10)^{a}	34 (7.3)^{d}	<0.27	<0.050	0.17 (0.07)^{a}
BC3P1.5	7.6 (0.20)^{a}	**5.9 (0.69)^{bc}**	**4.8 (0.12)^{c}**	0.10 (0.011)^{abc}	**46.5 (3.77)^{cd}**	**10.9 (0.55)^{cde}**	2.3 (0.20)^{a}	7.7 (0.03)^{a}	**7 (1.0)^{c}**	0.31 (0.08)	<0.050	0.21 (0.03)^{ab}
BC6P1.5	**8.5 (0.09)^{d}**	**7.7 (0.62)^{d}**	**6.7 (0.44)^{de}**	0.11 (0.005)^{abc}	**62.1 (4.10)^{ef}**	**10.6 (0.19)^{bcd}**	2.2 (0.10)^{a}	7.9 (0.06)^{a}	**3 (0.5)^{ab}**	<0.27	<0.050	0.24 (0.03)^{ab}
BC0P3	7.8 (0.36)^{abc}	4.9 (0.35)^{ab}	**4.4 (0.48)^{bc}**	**0.12 (0.005)^{bc}**	**36.7 (2.72)^{bc}**	**12.0 (0.55)^{ef}**	2.2 (0.00)^{a}	8.0 (0.10)^{a}	34 (13.2)^{d}	<0.27	<0.050	0.22 (0.02)^{ab}
BC3P3	**8.3 (0.03)^{cd}**	**6.5 (0.13)^{cd}**	**5.6 (0.48)^{cd}**	0.11 (0.002)^{abc}	**51.3 (5.45)^{de}**	**11.8 (0.27)^{def}**	2.2 (0.10)^{a}	7.8 (0.16)^{a}	**7 (0.4)^{c}**	<0.27	<0.050	0.21 (0.01)^{ab}
BC6P3	8.1 (0.13)^{bcd}	**7.7 (0.86)^{d}**	**8.0 (0.09)^{e}**	**0.12 (0.009)^{c}**	**65.0 (4.54)^{f}**	**12.3 (0.12)^{f}**	2.3 (0.10)^{a}	7.9 (0.03)^{a}	**4 (0.1)^{bc}**	<0.27	<0.050	**0.28 (0.02)^{b}**

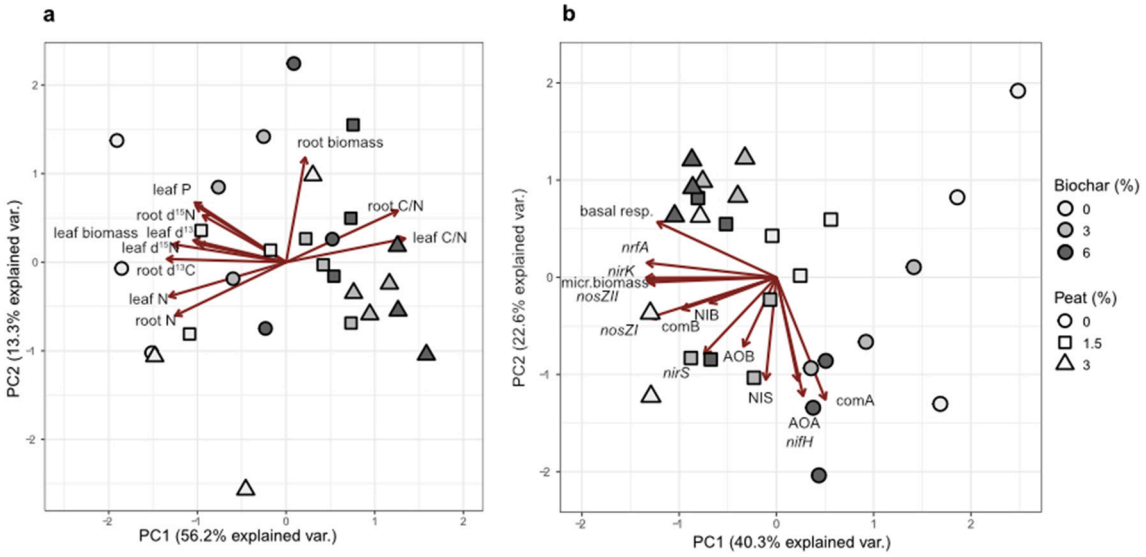

Fig. 1. Principal component analysis (PCA) biplot illustrating the response pattern of (a) plant and (b) microbial properties in a metal- and PAH contaminated soil treated with biochar- and peat in an outdoor field trial. The shapes indicate the level of peat treatment; their fill indicates the level of added biochar. The direction and the length of the fitted vectors are proportional to the correlation between the two PCs and each response variable.

and 280 g m^{-2} when 6 % biochar was added (Fig. 2a; Supplemental Table S5). Aboveground grass biomass was not affected by peat amendment or the interaction between peat and biochar, and root biomass was unaffected by both biochar and peat treatments (Fig. 2b). Amendment with biochar also decreased the N content in plant leaves, but even stronger when combined with peat, while peat alone had no effect (Fig. 2c). To a lesser extent, root N was also decreased by biochar (Supplemental Fig. S3). Following the decline in plant N content, the C/N ratios in plant leaf and root significantly increased in treatments with biochar and peat, whereas leaf P decreased (Supplemental Fig. S3; Supplemental Table S5). Grass biomass had a significant negative correlation with the increase in soil pH, TOC and C/N (Supplemental Fig. S1).

Leaf $\delta^{15}N$ signatures were highest in the control treatment, 9.3 ± 0.5 ‰, and significantly decreased with amendment of peat alone to 6.1 ± 0.4 ‰ and biochar alone to 7.0 ± 0.02 ‰ (Fig. 2d). Combined treatments of 3 % peat and 6 % biochar had the lowest leaf $\delta^{15}N$, reaching 4.8 ± 0.3 ‰.

Root $\delta^{15}N$ values were only significantly affected by peat amendment (Supplemental Fig. S3e), decreasing from 7.5 ± 0.5 ‰ in the control soil to 4.7 ± 0.2 ‰ in the treatment with 3 % peat. Soil $\delta^{15}N$ also significantly decreased with addition of biochar and peat but this decline was only up to 1 ‰ from the control, thus smaller compared to effects on root and leaf $\delta^{15}N$ (Supplemental Fig. S3f). The leaf, root and soil $\delta^{15}N$ values were significantly negatively correlated with soil N, CEC and TOC (Supplemental Fig. S1).

3.4. Microbial biomass, respiration and physiological response

Amendment with either biochar or peat alone increased microbial biomass (16S rRNA gene copy number) by 60 % and 100 % compared to the control soil (Fig. 3a), but the effect of peat was stronger than that of biochar (Supplemental Table S5). However, addition of both amendments did not result in an additive increase in 16S rRNA gene abundance. Basal respiration was also significantly affected by peat addition, increasing by 83 % in soil amended with 3 % peat compared to controls (Fig. 3b). In contrast, the effect of biochar on basal respiration was less pronounced, as only a weak, yet still significant overall increase was detected (Supplemental Table S5). Microbial biomass and basal respiration were positively correlated with each other, as well as with soil TOC and CEC, yet negatively with leaf N and $\delta^{15}N$ (Supplemental Fig. S2). Basal respiration, but not microbial biomass, was also positively

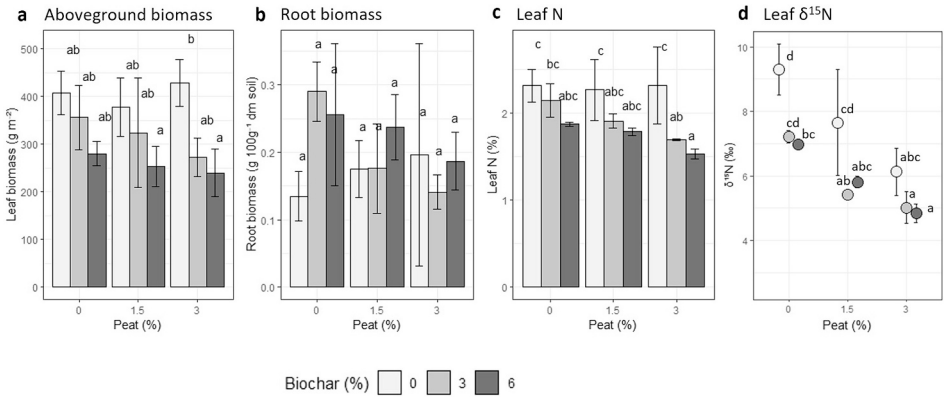

Fig. 2. Effects of biochar and peat amendments on (a) aboveground biomass (leaf and stalks), (b) root biomass, (c) leaf nitrogen (N) content and (d) leaf $\delta^{15}N$ in a field trial with the grass *Lolium perenne* growing in a PAH- and metal contaminated soil. Bar- and dotplots show means ± standard deviation for $n = 3$. Letters indicate significant differences (Tukey's honestly significant differences test, $P < 0.05$) between means for each treatment. Leaf N and $\delta^{15}N$ values were log10 transformed prior to statistical testing. Results of two-way ANOVA tests are provided in Supplemental Table S5.

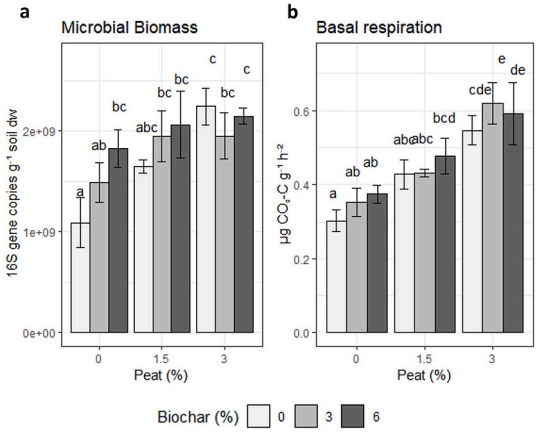

Fig. 3. Effects of biochar and peat amendments on a) 16S rRNA gene copies, a proxy for microbial biomass and b) soil basal respiration in a field trial with a PAH- and metal contaminated soil. Barplots show means ± standard deviation for n = 3. Letters indicate significant differences (Tukey's honestly significant differences test, $P < 0.05$) between means for each treatment. Results on the respiration of added C substrates are provided in Supplemental Fig. S4, and results of the two-way ANOVA tests are provided in Supplemental Table S5.

correlated with soil pH.

The peat addition increased respiration of all added carbon sources and it increased with a higher peat application rate (Supplemental Fig. S4). Application of biochar, on the other hand, increased respiration from most of the carboxylic acids, but decreased respiration from most of the sugars, and did not affect respiration from the amino acids. The ordination plots showed that the rate of peat addition largely explained the separation of treatments along the first PCO component, explaining almost 55 % of the variation in the physiological response (Supplemental Fig. S5). The substrates contributing most strongly to this separation were the nitrogen compounds and ascorbic acid. The separation along the second PCO component mainly reflected the rate of biochar addition and explained almost 35 % of the variation. The sugars and oxalic acid were the main contributors to this separation. The redundancy analysis revealed that physiological response of the microbial communities was mainly due to changes in soil water holding capacity (28 %) and CEC (21 %), and to the grass biomass (17 %), with nutrient and contaminant concentrations and pH in the soil water contributing less (Supplemental Fig. S6).

3.5. Microbial N cycling guilds

3.5.1. Microbial guilds involved in nitrification

In the control soil, AOB were the dominant ammonia oxidizers, being about one order of magnitude more abundant than AOA (Fig. 4a and b). The AOB were highest in soil amended with 3 % peat only, yet they decreased to similar levels as the control soil when amended with biochar. The addition of 6 % biochar increased the AOA abundance twofold compared to the control, but in treatments with increasing amounts of peat the effect of biochar on the AOA abundance was negligible (Fig. 4a). The genus *Nitrospira* was the dominant nitrite oxidizing bacteria, being 50 times more abundant than *Nitrobacter* (Fig. 4c and d). Similar to the AOB, the abundance of *Nitrobacter* increased twofold by the addition of 3 % peat in the absence of biochar, whereas no effect of either amendment was observed on *Nitrospira* abundances (Supplemental Table S5). Among the complete nitrifiers, the abundance of the comammox clade 'A' (comA) was about one order of magnitude higher among all treatments compared to the clade 'B' (comB; Fig. 4e and f). Addition of peat in general resulted in a significant decrease in comA abundance, whereas comB abundance increased and both nitrifiers showed no significant effect of biochar additions (Supplemental Table S5). The AOB, *Nitrospira* and comA abundance were positively correlated with leaf N, and comA also with leaf $\delta^{15}N$ whereas *Nitrospira* abundance correlated negatively with root biomass (Supplemental Fig. S2).

3.5.2. Microbial guilds involved in nitrate ammonification, denitrification and N_2O reduction

The *nirK*-type denitrifiers dominated over the *nirS*-type by a factor four in the control soil, increasing almost threefold in abundance with the addition of peat with or without biochar (Fig. 4h). A weak overall effect of biochar on both *nirK* and *nirS* abundances was also observed (Supplemental Table S5). For the N_2O reducers, *nosZII* was about twice as abundant as *nosZI* in control soil, and both communities increased with either the addition of biochar or peat (Fig. 4i and j). Similar to the 16S rRNA gene abundance, the effect of one amendment type was only evident in the absence of the other. No increases were observed in either *nosZI* or *nosZII* abundances at intermediate and high peat and biochar amended soils. The response pattern of nitrate ammonifiers, as determined by *nrfA* abundance, was similar to that of *nosZII* and *nirK*-type denitrifiers, in that peat and biochar addition resulted in significantly increased abundance (Fig. 4j) and were positively correlated to microbial biomass and basal respiration as well as soil TOC and CEC. By contrast, denitrifier- and nitrate ammonifier abundances were mostly negatively correlated with plant responses such as leaf biomass, N and $\delta^{15}N$ (Supplemental Fig. S2b).

3.5.3. Microbial guilds involved in nitrogen fixation

The abundance of N_2-fixing bacteria decreased overall in response to peat addition, with no clear general effect of biochar (Fig. 4k). Treatment with 6 % biochar without peat had about a 50 % higher abundance of N_2-fixers compared to treatment with 3 % peat and no biochar, otherwise there were no significant differences between the treatment groups (Fig. 4k). There were no correlations of *nifH* with soil and plant factors.

4. Discussion

4.1. Immobilization of N

The negative effect of the biochar additions on plant biomass and plant N content (Fig. 2) suggests that biochar reduced plant N availability and that the added compost, inorganic N fertilizer and peat could not compensate for this effect. It is likely that this was mainly caused by increased N immobilization into microbial biomass as supported by the significantly increased 16S rRNA abundance and increased basal respiration (Fig. 3). We cannot rule out that abiotic immobilization of NO_3^- and NH_4^+ occurred onto the surface of the biochar (Clough et al., 2013; Gao et al., 2019), or that the biochars' pH altered nutrient availability or uptake by plants (Barrow and Hartemink, 2023). However, the similar levels of microbial biomass observed in biochar and peat amended soil despite substantial differences in plant biomass and N suggests that microorganisms competed more effectively for N compared to plants in response to biochar amendment, and any potential abiotic sorption of N onto the biochar did not limit their growth whereas plant growth and N uptake was restricted.

Addition of C-rich substrates such as biochar and peat will stimulate microorganisms to scavenge for N and P (Cleveland and Liptzin, 2007), thereby immobilizing these nutrients in their biomass. When biochar is used as a sole amendment, biochar-labile C may be most responsible for microbial N immobilization (Lehmann et al., 2003). The biochar used here is wood-based and produced at a higher temperature (750 °C), and therefore likely contains only a low amount of biochar-labile C (Downie et al., 2012; Nguyen et al., 2010) and thus predicting that N immobilization, will be low (Zimmerman et al., 2011). Instead, biochar amendment may have increased the habitat suitability for microorganisms in a more general way in this study, for instance by providing a greater surface area and porosity and increasing soil pH (Zimmerman, 2010).

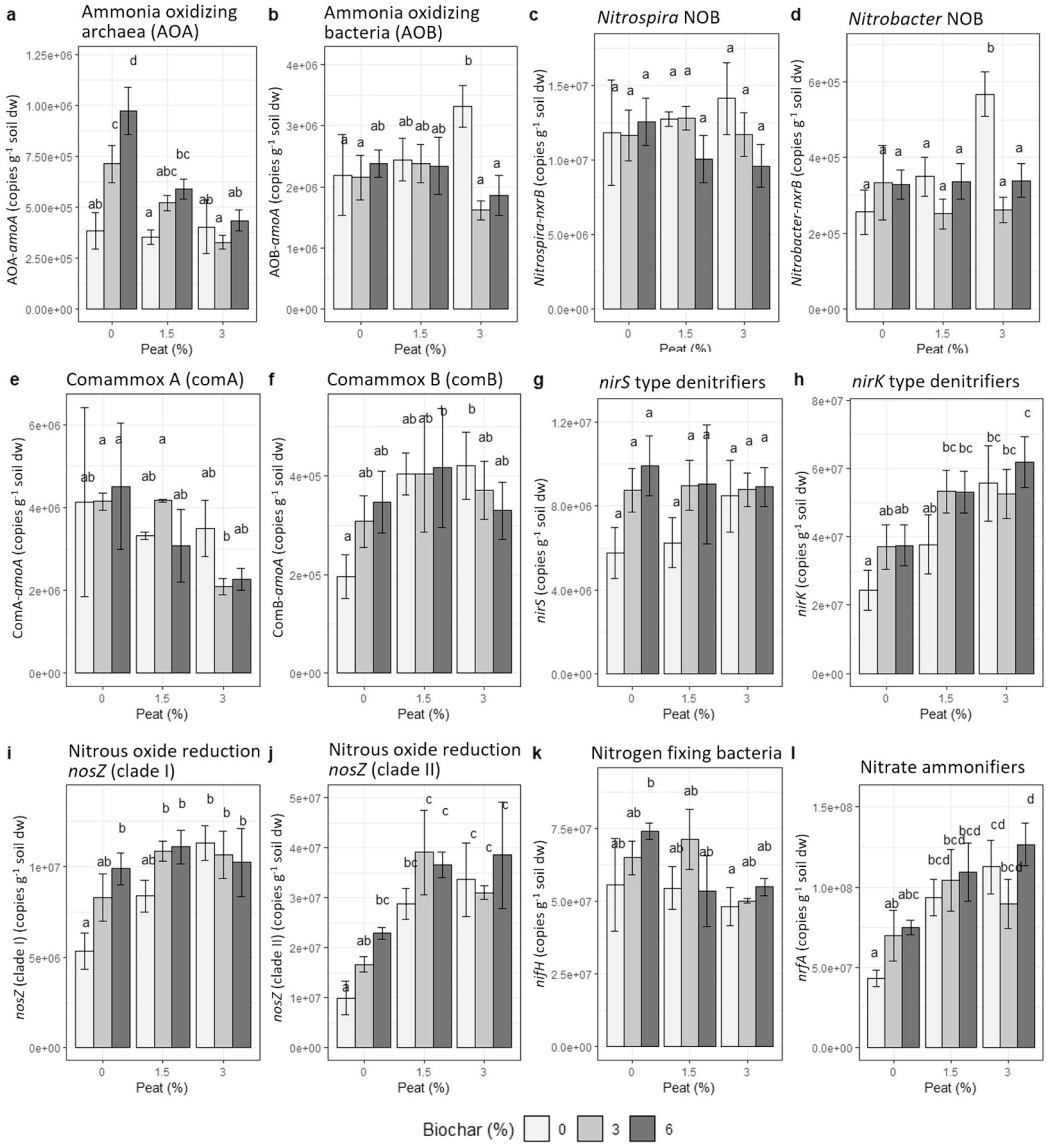

Fig. 4. Effects of biochar and peat amendments on N cycling microbial guilds in a field trial with a PAH- and metal contaminated soil. Barplots show means ± standard deviation for n = 3. Letters indicate significant differences (Tukey's honestly significant differences test, $P < 0.05$) between means for each measure. Abundances of comA were reciprocally transformed and *nosZII* were log10 transformed prior to statistical testing.

Indeed, the surface area of our biochar was relatively high compared to other biochars (Sun et al., 2014). The finding that microbial biomass did not further increase with biochar in peat-amended soil, suggests that the positive effect of biochar on microbial biomass is higher in soil with a low SOC, which seem to be in line with previous studies (Li et al., 2020). Basal respiration increased with biochar regardless of peat levels, suggesting that biochar stimulated the co-metabolization of native soil organic matter and/or compost independent of added peat. For the peat-amended plots, it is likely that the increase in basal respiration was caused by the mineralization of peat (Bridgham et al., 1998), which will decrease SOC stocks over time (Vestberg et al., 2009).

4.2. Stable isotopic patterns

The higher $\delta^{15}N$ of leaves, roots and soil in the control soil compared to the amended soil suggest that gaseous losses of N were larger in the control plots (Craine et al., 2015; Högberg, 1997; Högberg and Johannisson, 1993). Thus, the $\delta^{15}N$ data aligns with the increased N immobilization in response to the amendments, in line with the results on plant biomass, leaf N content and microbial biomass. This finding also agrees with a previous biochar field study that reported a lower leaf $\delta^{15}N$ and N immobilization (Asadyar et al., 2021; Bai et al., 2015).

Besides a reduction of gaseous N losses, isotopic effects may also occur through various other mechanisms, such as fractionation during plant N uptake, $\delta^{15}N$ values of the primary N sources, and isotopic enrichment of microbial biomass. However, plant available N was low and decreased further due to microbial immobilization of N in response to the treatments. During such conditions isotopic fractionation during N-uptake is minor (Högberg et al., 1999) and the 5 ‰ higher leaf than bulk soil $\delta^{15}N$ in the control reflects a relatively ^{15}N enriched plant N source in this treatment (Fig. 2, Supplemental Fig. S3). Biochar and peat are depleted in ^{15}N compared to the control soil (Supplemental Table S2). While the N content of biochar is low and probably not easily mineralizable and accessible to plants, mineralization and uptake of N from peat may partially explain the lower leaf $\delta^{15}N$ in peat-amended soil. But when peat and biochar are combined, plant N showed to be even stronger restricted compared to soil that was only treated with biochar; hence plant uptake of peat-derived N in the presence of biochar seems unlikely. Alternatively, the increasing microbial biomass was preferentially using relatively ^{15}N enriched N for their biomass growth,

leaving the remaining plant available N relatively ^{15}N depleted (Collins et al., 2008; Craine et al., 2015; Dijkstra et al., 2008). The higher microbial biomass in peat- and biochar amended soil, immobilizing soil N and potentially reducing gaseous losses, seems therefore the most plausible explanation for the lower $\delta^{15}N$ values. This is also supported by the significant negative correlation between leaf $\delta^{15}N$ and the microbial biomass (Supplemental Fig. S2b).

We cannot decide which gaseous N loss pathway, denitrification or ammonia volatilization, if any, could be most reduced when biochar and peat were added. Although denitrifying guilds increased with both amendments at the field trial, the depleted $\delta^{15}N$ isotopic patterns of plants contradict any potential increase in denitrification activity. Conditions for denitrification are probably not optimal during dry summer periods (Lennon and Houlton, 2017), but since denitrifiers are generally facultative anaerobes they could still thrive and grow while respiring oxygen. Higher abundance of *nirS*-type denitrifiers were previously related to an enrichment of soil $\delta^{15}N$ and low soil NO_3^- (Lennon and Houlton, 2017), but our result suggest that at more local and/or short time scales, and with added soil amendments, other processes may influence this relation. Indeed, denitrification can be decreased in biochar-amended soil (Ameloot et al., 2016; Borchard et al., 2019). Microbial or physical N immobilization was found most responsible for this reduction (Case et al., 2012). In addition, biochar has the potential to reduce NH_3 volatilization (Taghizadeh-Toosi et al., 2012) but the effect is context-dependent (Sha et al., 2019). Indeed ammonia volatilization may have occurred in our non-amended control soil, given the higher pH of 7.7 and low OC content (Zhenghu and Honglang, 2000). Thus, although we cannot point out which N loss pathway was reduced most, the $\delta^{15}N$ depletion in plants is strong evidence that biochar and peat amendment has led to a smaller N available pool from which less general gaseous N losses occurred.

4.3. Alterations to microbial N cycling guilds

Peat treatments altered the N cycling community more than biochar and also modified the response to biochar, reflecting the influence of soil edaphic properties and nutrient status (Fig. 4). The increased abundance of AOB, *Nitrobacter* nitrite oxidizers, comB-type complete nitrifiers, *nirK*-type denitrifiers, *nosZII* and nitrate ammonifiers in peat compared to biochar (Fig. 5) could be either an effect of a higher addition of labile N and other nutrients, or that these N cycling communities were present in the peat, thereby inoculating the soil with N cycling communities. The effects of biochar and peat on the nitrifying guilds showed a variable pattern, which may be largely explained by altered nutrient conditions. For instance, AOA are thought to have an advantage over AOB in environments with lower pH and NH_3 availability, whereas AOB are more neutrophilic and favored under conditions with higher NH_3 availability (Hink et al., 2018; Prosser and Nicol, 2012). This may explain the increased in AOA with biochar amendment in soil without peat amendment, in line with the low plant-available N and the idea that the N-cycling is more balanced (i.e., N losses are reduced) when biochar is the sole soil amendment, as supported by the $\delta^{15}N$ measurements. Likewise, altered nutrient conditions may also explain the responses of nitrite oxidizing communities. The *Nitrobacter* nitrite oxidizers, like the AOB, are mainly adapted to environments with high substrate (NO_2^-) availability whereas *Nitrospira* are favored at lower NO_2^- levels (Nowka et al., 2015; Schramm et al., 1999). This is in line with the increased total N observed with the addition of peat, as both AOB and thereby the transformation of NH_4^+ to NO_2^- may have had a more positive effect on the *Nitrobacter* compared to the *Nitrospira*. Little is known about the potential niche differentiation of comammox bacteria groups (Koch et al., 2019). It has been suggested that some comammox clade A bacteria are adapted to oligotrophic habitats (Kits et al., 2017; Sakoula

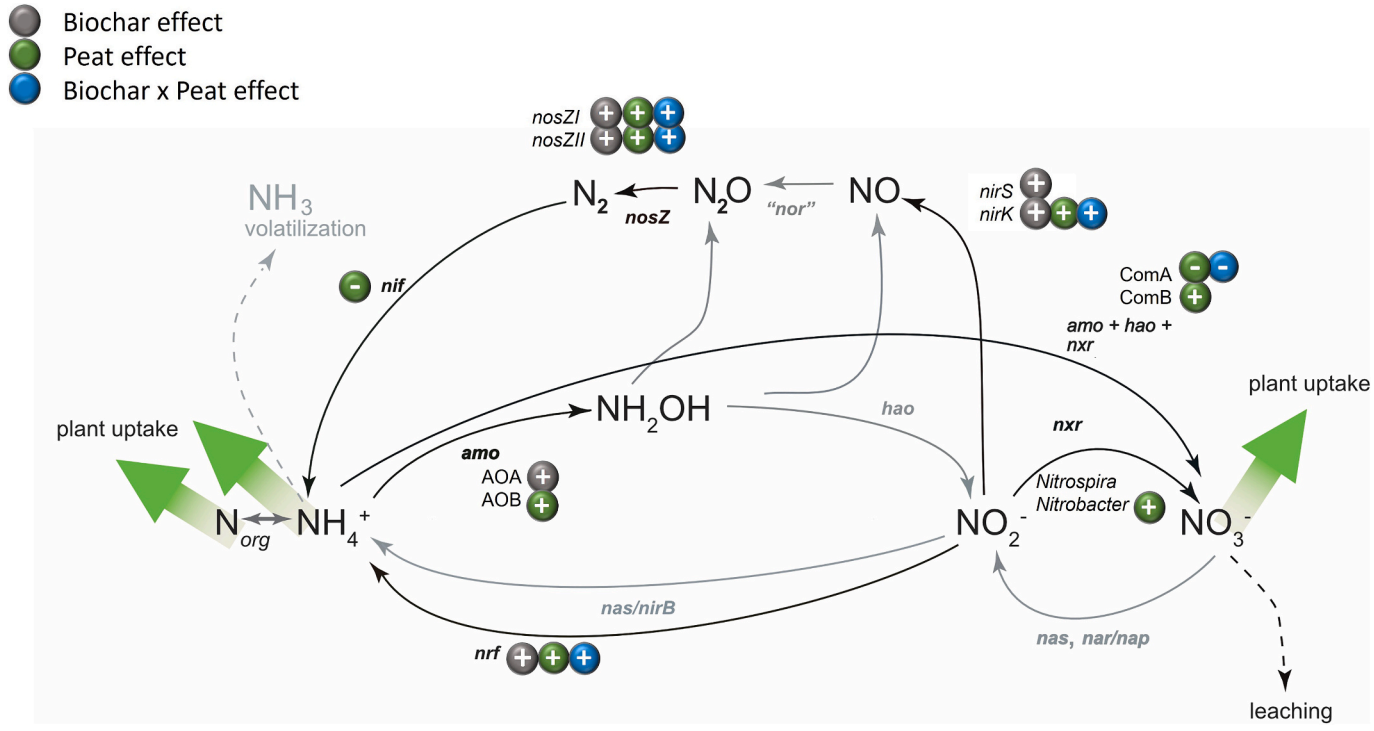

Fig. 5. Summary on the effects of biochar and peat amendments on microbial N cycling guilds in a field trial with a PAH- and metal contaminated soil. Pathways and marker genes included in this study are presented in black, other pathways and genes not measured in this study are shown in grey. Significant effects on the abundance of the marker gene or specific genus compared to the control treatment are presented by a plus or minus in dots in grey (biochar), green (peat) and blue (combined effect of biochar and peat). An increased abundance is a measure of increased capacity for that transformation but not a direct measure of activity. Effects were indicated by a two-way ANOVA and post-hoc Tukey's honestly significant differences test. The overview of the N cycling microbial guilds is based on a previous figure (Hallin et al., 2018).

et al., 2021), while clade B are more variable, thriving in acidic forest soils (Pjevac et al., 2017; Shi et al., 2018) as well as arable soils with higher NH_3 and pH (Lin et al., 2020). This was also reflected in the increase of clade B, and concurrent decrease of clade A, in the peat amended soil with higher N.

In contrast to the nitrifying guilds, the response of the heterotrophic denitrifying and nitrate ammonifying guilds to biochar and peat amendment was mostly positive, especially for peat, and were highly correlated to the increase in microbial biomass and basal respiration. This suggests that the increase in these guilds may rather be connected to the increased suitability of amended soil as a habitat for heterotrophic microorganisms supported by addition of C substrates. Nitrate ammonifying bacteria are known to increase under high C/N conditions (Rütting et al., 2011; Schmidt et al., 2011), which could explain that they became more abundant than the denitrifiers with the additions of biochar and peat. In theory, a dominance of organisms performing nitrate ammonification over denitrification should result in lower rates of N-loss through gaseous N emissions. While this would be in line with the lower $\delta^{15}N$ values observed in the amended soils, we can only speculate as to the relative importance of each guild in this system as organisms that can perform nitrate ammonification may also produce and consume N_2O (Saghaï et al., 2023; Stremińska et al., 2012). Indeed, the increases in abundance of *nosZI* and *nosZII* N_2O reducing communities indicated an increased capacity for N_2O consumption, in response to peat and biochar addition, thereby providing a potential for decreased emissions of this important greenhouse gas. This is in line with previous work showing that addition of biochar can significantly reduce ratios of N_2O production to total denitrification ($N_2 + N_2O$) in soils (Cayuela et al., 2013; Harter et al., 2014; Kaur et al., 2023) which may be linked with shifts in N_2O reducing community abundance and structure (Harter et al., 2017; Zhang et al., 2021). The *nosZ* guilds have also shown higher abundance when pH increases (Dörsch et al., 2012; Jones et al., 2014) which was also found in our study.

4.4. Biochar and peat amendment and their role in reducing potential microbial toxicity

Soil contaminant levels may have had a potential toxic effect in untreated soil, although the effect is probably small and lies below or around EC10 levels (concentration giving 10 % effect in single species toxicity experiments). Total levels of Cu corresponded to EC10 levels for effects on N cycling guilds in aged soils under planted outdoor conditions (Rijk et al., 2023). Toxicity effects of Cu on soil microorganisms and Zn effects on plants were also indicated by the soil-specific bioavailability-corrected HC25 values (hazardous concentration for 25 % of the species) obtained with the Threshold calculator v3.0 (ARCHE, 2020b) (Supplemental Table S6). These HC25 values were also derived with EC10 values, therefore probably signaling small effects in both untreated and treated soil, but even expected to be lower in treated soil. Total levels of PAH were also in the same range as published EC10 values for general microbial toxicity (Cheng et al., 2014) as well as to changes in N cycling guilds (Yi et al., 2022). However, since these experiments were performed with spiked soils, lower toxic responses are expected in historically contaminated, aged soil (Eom et al., 2007), and soils with plants (Joner et al., 2001; Yang et al., 2007).

It is challenging to disentangle effects of substrate addition and altered soil physicochemical properties from potential reduced toxicity of immobilized contaminants. The shifts in microbial biomass, respiration and N cycling communities, were more strongly related to peat amendment (Fig. 1b) and correlated with abiotic properties (Supplemental Fig. S1), while peat had only a small effect on PAH and metals concentrations in the soil solution and metal content in ryegrass (Enell et al., 2020). Also, the physiological response of the microbial communities was most closely linked with changes in general soil properties (Supplemental Fig. S6) such as water holding capacity and CEC, important aspects of soil as a space for microbial communities, and to plant growth providing inputs of fresh organic matter serving as C source for the microorganisms. This seems to be in line with other studies that investigated biochar and other organic amendments in contaminated soils, finding that soil abiotic properties and N status predominantly influenced responses of microbial N cycling guilds (Li et al., 2019; Li et al., 2021; Zhao et al., 2020). Furthermore, comparable patterns of changes in microbial N cycling guilds as in our field trial were found in meta-analyses of studies on unpolluted soils (Xiao et al., 2019; Zhang et al., 2021).

As the plant growth decreased with a concomitant immobilization of contaminants following biochar amendment, the effect of decreased plant toxicity is probably a minor effect, compared to effects of nutrient immobilization. Also, we cannot exclude that the pH increase in biochar-amended soil might have impacted plant growth and nutrient uptake, shifting it further outside the optimum pH range of 5.5–7.5 for perennial ryegrass (Hannaway et al., 1999). Hence, the results of our field trial underscore the need for adapted nutrient management to compensate for nutrient immobilization and ensure successful plant cover establishment and health (Beesley et al., 2011).

5. Conclusions

The application of a wood-based, high temperature biochar and a common garden peat to a metal- and PAH- contaminated urban soil resulted in an overall increase in soil microbial biomass and respiration. The response of N cycling communities to these soil amendments showed the most consistent increases of denitrifying guilds (*nirK*, *nosZI* and *nosZII*) and microorganisms involved in nitrate ammonification (*nrfA*), while more variable responses were found among nitrifying guilds. The lower $\delta^{15}N$ values in plants after biochar and peat amendment suggest decreased N gaseous losses by either denitrification or ammonia volatilization, despite increase in corresponding microbial denitrifying guilds. This seems to mainly be a result of biotic N immobilization in the microbial biomass as soil solution concentrations of NO_3^- and NH_4^+ were negligible during the growing season. A decreased plant biomass, plant N and increased microbial biomass also supports the view of microbial N immobilization. This shows that the remediation of contaminated soil with biochar and peat amendments, apart from contaminant immobilization, has important co-benefits, as it increased the capacity of microbial functions and may decrease negative impacts on ecosystems by N gaseous emissions. While the sorption effectiveness and long-term performance of these amendments should be decisive for lowering risks for human health and the environment, our data provides proof that soil remediation with biochar and peat may be an effective method to also restore soil ecological functioning and improve soil health in contaminated soil.

CRediT authorship contribution statement

Ingrid Rijk: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Alf Ekblad:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition. **A. Sigrun Dahlin:** Writing – review & editing, Visualization, Methodology, Formal analysis. **Anja Enell:** Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Maria Larsson:** Writing – review & editing, Investigation. **Prune Leroy:** Investigation, Formal analysis. **Dan B. Kleja:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Charlotta Tiberg:** Writing – review & editing. **Sara Hallin:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Christopher Jones:** Writing – review & editing, Visualization, Supervision, Methodology, Formal analysis.

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

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