Short Communication

Use of microdialysis to assess short-term soil soluble N dynamics with biochar additions

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A B S T R A C T

To date there has been little effort to assess fine-scale soil solution nitrogen (N) dynamics over time and space in response to biochar application within days. Herein we applied microdialysis to investigate the spatial and temporal dynamics of free amino acids (AA), ammonium (NH_4^+), and nitrate (NO_3^-) following biochar application to a column containing sandy loam forest soil. Wood biochar (diameter $\leq$ 5 mm) was applied either at surface or mixed through the entire soil column. Biochar stimulated localized soluble N diffusive fluxes, and the vertical distribution of AA and NH_4^+ hotspots gradually matched the distribution of biochar particles in the soil matrix over time. Increases in soil AA and NH_4^+ concentrations were more homogeneous along soil profile when biochar was mixed through the soil core and were more concentrated at the surface when biochar was surface applied. Increases in NO_3^- concentrations were only observed at surface soil layers following biochar addition regardless of the application strategy and generally exhibited a high degree of variation over the course of the experiment. Microdialysis sampling allowed for quantification of 'hotspots' of soil soluble N in association with the "charosphere" across time and space.

1. Main text

Prior studies of wood biochar application to forest soils have exhibited increased nitrification and mineralization rates at the plot scale (DeLuca et al., 2006; Gundale and DeLuca, 2007; MacKenzie and DeLuca, 2006); however, there have been few studies that effectively evaluate fine-scale changes in soluble N pools within the "charosphere" (soil immediately surrounding biochar) (Yu et al., 2019). Once biochar is applied to soil it can adsorb soluble organic materials, stimulate nutrient transformations, stimulate microbial growth, increase gas and nutrient exchange, influence water movement, and form complexes with minerals (Lehmann and Kleber, 2015; Pingree and DeLuca, 2017). Consequently, *charosphere* soil can be directly affected by biochar properties and may demonstrate different responses compared to bulk soil (Quilliam et al., 2013). Standard procedures to assess soil N availability are based on batch extraction of soil that give a "snapshot" estimate of free amino acids (AA), available ammonium (NH_4^+), and nitrate (NO_3^-). Although batch extractions are efficient and widely used, they introduce a number of artifacts including the elimination of microsite and spatial influences and do not effectively describe rates of N pool turnover (Inselsbacher, 2014; Inselsbacher and Näsholm, 2012). Microdialysis probes were originally developed for use in neuroscience, but more recently have been applied to soil science research (Brackin

et al., 2015; Demand et al., 2017; Inselsbacher et al., 2011; Shaw et al., 2014). The small probes cause minimal disturbance to soil structure and the passive diffusion of soil solutes across a semi-permeable membrane allows dialysate to be collected over time without re-taking samples allowing "real-time" evaluation of fine scale nutrient dynamics (Brackin et al., 2017; Buckley et al., 2017). Therefore, microdialysis has the potential to provide higher spatial and temporal resolution for understanding short-term solution N chemistry in the *charosphere* (Hill et al., 2019).

Microdialysis was used to monitor fine scale concentrations or diffusive flux rates of AA, NH_4^+, and NO_3^- along a soil profile following biochar addition to forest soil over a 16 day period. We hypothesized that the diffusion flux rate of NO_3^- will be accelerated by biochar addition whereas AA and NH_4^+ will remain unchanged as per previous field and laboratory findings (DeLuca et al., 2006; Gundale and DeLuca, 2007). To further refine our hypothesis and examine the spatial distribution of soluble N hotspots in response to biochar across time, we compared two biochar application strategies, biochar applied to surface soil only and mixed through the entire soil core.

Soils were collected from the A horizon of an undisturbed forest site (Lubrecht Experimental Forest, Greenough, MT, USA, 46°53′32″ N, 113°23′03″ W). The granitic sandy loam soil (Typic Haplocryepts) had a pH of 6.5, total C content of 16.5 g C kg^{-1} soil, total N content of

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$1.4\,g\,kg^{-1}$, extractable AA, NH_4^+ and NO_3^- concentration of 2.15 mg AA-N kg^{-1}, 5.56 mg NH_4^+-N kg^{-1}, and 0.68 mg NO_3^--N kg^{-1}. Biochar was produced using charred wood waste from lumber mills of F.H. Stoltze Land & Lumber Company (Columbia Falls, MT, USA) as a by-product from the electrical co-generation plant. Biochar was press processed and sieved to 5 mm for application. The biochar had a pH of 7.8, total C content of $595\,g\,C\,kg^{-1}$, total N content of $0.8\,g\,kg^{-1}$, 0.14 mg N kg^{-1} NH_4^+, and both AA and NO_3^- concentrations were too low to be detected. Soils were pre-moistened to approximately 60% field capacity, passed through a 2-mm sieve, packed into 6.5 cm diameter by 33.5 cm length PVC column with gentle compaction that resulted in an evenly distributed soil profile. The resulting "control" soil column (no biochar) had a bulk density of $1.0\,g\,cm^{-3}$. Treatment columns were prepared by applying 2% (w/w) biochar at surface only (mixed into top 0–3 cm with gentle compaction) or mixing biochar with soil for the entire column (0–33.5 cm) during packing. Each column had three small holes (by 1 mm diameter drill bit) bored at depths of 1, 6, 12, 18, 25, and 33 cm to allow access by the microdialysis probe. Each treatment or control column represented an individual unit and was replicated six times. The columns were stored at room temperature over the course of the experiment. Water loss by evaporation was minimized by covering the columns with cellophane and were not compensated during the experiment to eliminate the impact of drying-rewetting or leaching with water addition. A schematic experimental design is given in Fig. S1.

Microdialysis probes used in our study had 10 mm membrane length, 500 μm membrane diameter with a 20 kDa molecular weight cutoff (CMA 20, Harvard Apparatus). To sample solution on a fine scale, probes were inserted into soils through side holes in the columns by using the needle and introducer supplied by the manufacturer. The perfusate flow rate was set to $5\,\mu L\,min^{-1}$ according to Inselsbacher et al. (2011) and Shaw et al. (2014). Dialysates were collected over time periods of 2 h in 1.5 mL microfuge tubes. A total of 18 samples were collected for an individual column (3 samples per depth x 6 depths). All dialysates were first sampled 24 h following biochar addition (day 1), and were then sampled every five days for a total of 16 days (day 6, day 11, day 16). Calibration of the probes was carried out before sample collection on each sampling day according to Inselsbacher et al. (2011) and the relative recovery was found to remain stable (28.3 ± 4.9%) across all N forms. Probes were run outside of the soil column for 15 min to ensure that the initial empty volume in the tubes had been flushed before sampling. Dialysate NH_4^+ and NO_3^- were determined colorimetrically following Mulvaney et al. (1996) and Miranda et al. (2001). Total dialysate AA was determined by the *o*-phthadialdehyde spectrofluorometric method according to Jones et al. (2002). The concentrations were converted to flux rates (amount arriving per unit surface area of the probe per hour) before analyses.

Each column was treated as an analysis unit meaning that three measurements made within each column at a given soil depth on the same sampling day were averaged in order to generate column level values. Data were first analyzed using a factorial ANOVA to reveal the significant effect of sampling time and biochar application strategy on soluble N responses at different ranges of soil depths. Mean values and 95% confidence intervals for AA, NH_4^+, or NO_3^- flux rates at specific depth and at specific sampling day were then calculated and compared across treatments and controls to reveal spatial and temporal patterns. All data were analyzed using R Studio.

Consistent with our expectations, localized soil AA, NH_4^+, or NO_3^- fluxes showed dynamic spatial and temporal patterns in response to wood biochar application regardless of whether the biochar was surface applied or mixed in the soil (Table 1, Figs. 1–3). For AA and NH_4^+, the positive effect of biochar tended to be more pronounced later in the experiment, where the vertical distribution of AA and NH_4^+ hotspots gradually matched the distribution of biochar particles in the soil matrix over time (Figs. 1 and 2). At day 16, soils with biochar exhibited nearly twice the flux rates of AA and NH_4^+ in the top layers (i.e.

0–6 cm) under either application strategy, where the surface application of biochar seemed to concentrate more AA and NH_4^+ in upper portions of the soil while the mixing strategy distributed them more evenly throughout the column (Figs. 1 and 2), potentially linked to the adsorption capacity of the wood biochar (Cheng et al., 2006; Chin et al., 2018; Gao et al., 2017, 2016; Gao and DeLuca, 2016).

Amino acids constitute an important source of N to both plants and microorganisms in soils (Schimel and Bennett, 2004) of the relatively N-poor forest ecosystems of the Inland Northwest. Our observations suggest that biochar yielded biotic or abiotic increases in the production rate of AA from polypeptides and alter the residence time of AA in surrounding microsite soils (Cheng et al., 2017; Hill et al., 2019). It is possible that biochar actively participated in constructing the zonal structure of soil organo-mineral complexes with protein coatings, where the proteinaceous material in soil solution was proposed to serve as a surface conditioner that added polar functionality to the hydrophobic surface of biochar, promoting the bonding interactions between biochar and mineral complexes (DeLuca et al., 2015; Keiluweit et al., 2010; Kleber et al., 2007). Similarly, biochar might directly facilitate a greater extent of NH_4^+ exchange in surrounding soils or indirectly alleviate the repression of mineralization through adsorbing high C:N ratio compounds that would otherwise induce microbial immobilization (Choromanska and DeLuca, 2001; Fujii et al., 2018). These findings are somewhat inconsistent with Liu et al. (2018) which showed that biochar had little effect on pool size or turnover rate of dissolved organic N, but supported by Gao et al. (2019) whose meta-analysis showed that wood biochar generally increased soil NH_4^+ by surface adsorption in non-field and short-term studies. It is also possible that biochar altered the *charosphere* microbial communities responsible for the changes in N transformations (Yu et al., 2019). It is also reasonable to expect that a 16-day lab trial would capture a more heterogeneous spatiotemporal pattern of *charosphere* AA diffusive flux changes immediately upon biochar application compared to longer-term field studies using bulk soil extractions. More importantly, the inconsistency of responses between *charosphere* soil and bulk soil highlighted the potential of microdialysis use in sampling and capturing fine-scale N hotspots.

The spatiotemporal pattern of NO_3^- hotspots (Fig. 3) suggests that NO_3^- was not sensitive to the biochar application strategy in most of the soil layers (i.e. 12–33 cm), except the top 0–12 cm layer where changes in NO_3^- clearly responded to surface biochar application (Table 1). This result is consistent with our hypothesis that we expected a close link between biochar and nitrification in forest soils. The intensive NH_4^+ hotspots close to biochar that were detected over the experiment and the relatively high background soil NH_4^+ concentration argue against substrate limitation of nitrification. The detection of NO_3^- hotspots in the *charosphere* compared to bulk soil (with a background NO_3^- of 0.68 mg kg^{-1}) supports the notion that the adsorption of high C:N ratio organic compounds or compounds inhibitory to nitrifying bacteria (Uusitalo and Smolander, 2008; Keech et al., 2005; White, 1994) might play a role in nitrification response to biochar. In our study, we also compared 2% and 5% (w/w) biochar rate, as well as coarse (5–10 mm in diameter) and fine particle size ($\leq$5 mm in diameter) biochar, but the 5% addition rate was found to result in similar patterns as the 2% rate and coarse biochar additions showed no treatment effect (data not shown).

The use of microdialysis to the assess biochar effects on soil solution N demonstrated: (1) Addition of wood biochar to a high C:N ratio, low NO_3^- forest soil increased the localized diffusive fluxes of amino acids, NH_4^+, and NO_3^-; (2) The spatial distribution of N hotspots along the soil profile dynamically responded to the biochar application strategy during a 16 day period. These findings can help us understand how biochar applications mediate changes in fine scale soil solution N chemistry in a temperate forest soil.

Table 1
The F and P statistics derived from analysis of variance (ANOVA) showing main and interactive effects of sampling time and biochar application strategy on soil free amino acids (AA), ammonium (NH_4^+), and nitrate (NO_3^-) diffusive flux rates sampled by a microdialysis system at multiple soil depth ranges in a 16-day incubation experiment. Significance levels: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, ns indicates $P \geq 0.1$.

| Localized N	Depth	Sampling time		Application strategy		Sampling time x Application strategy	
		F	P	F	P	F	P
AA	0–12 cm	112	***	13.1	***	3.60	*
	6–18 cm	79.7	***	0.12	ns	1.87	ns
	12–25 cm	100	***	9.52	**	8.37	**
	18–33 cm	39.6	***	11.1	**	4.66	*
	25–33 cm	19.6	***	5.87	*	3.33	ns
NH_4^+	0–12 cm	139	***	33.3	***	4.59	*
	6–18 cm	211	***	9.29	**	5.31	*
	12–25 cm	201	***	3.03	ns	3.83	ns
	18–33 cm	110	***	0.12	ns	0.18	ns
	25–33 cm	56.0	***	1.88	ns	0.03	ns
NO_3^-	0–12 cm	88.4	***	4.66	*	0.29	ns
	6–18 cm	18.8	***	0.14	ns	1.39	ns
	12–25 cm	54.66	***	0.02	ns	0.09	ns
	18–33 cm	9.47	***	4.79	*	0.89	ns
	25–33 cm	11.69	***	0.37	ns	0.06	ns
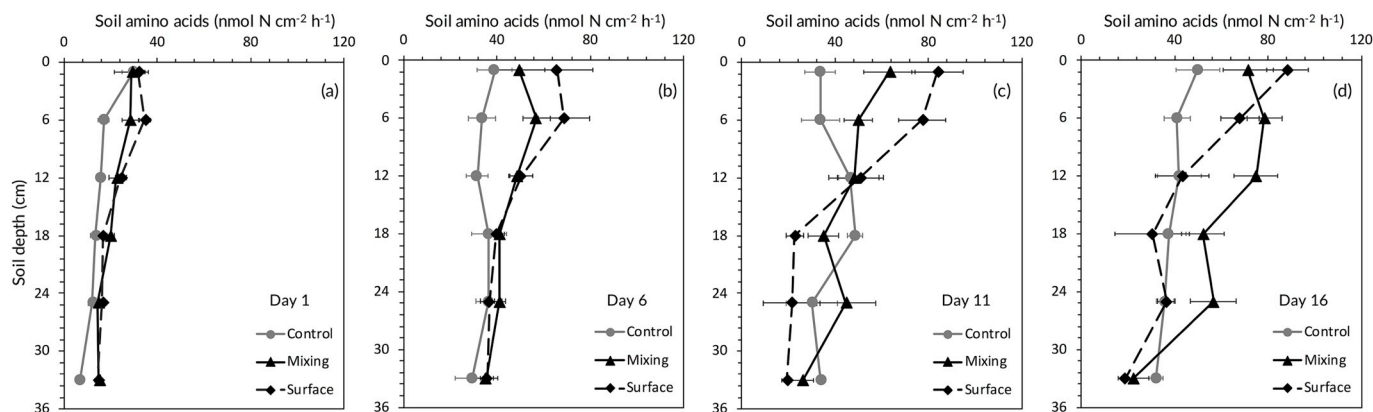
Fig. 1. Total free amino acids (AA) diffusive flux rates (nmol cm^{-2} h^{-1}) sampled by using microdialysis at varied depths in a soil column amended with no biochar (control), biochar mixed through the soil column (mixing), or surface addition of biochar (surface) in an incubation experiment at (a) Day 1, (b) Day 6, (c) Day 11, and (d) Day 16. Error bars represent 95% confidence interval for each data point (n = 6). Treatments were considered significantly different from each other when confidence intervals do not overlap at a given depth.
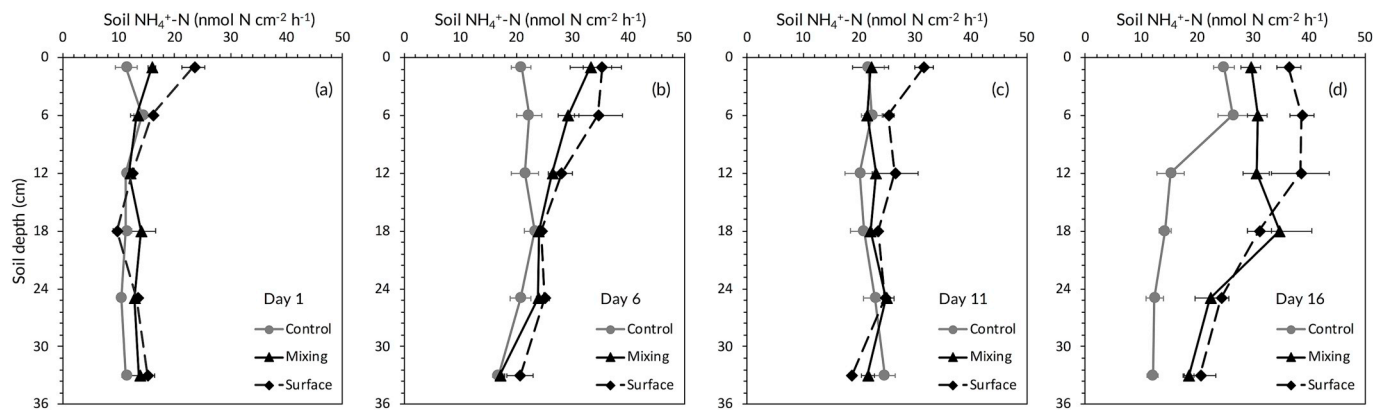

Fig. 2. Soil ammonium (NH_4^+) diffusive flux rates (nmol cm^{-2} h^{-1}) sampled by using microdialysis at varied depths in a soil column amended with no biochar (control), biochar mixed through the soil column (mixing), or surface addition of biochar (surface) in an incubation experiment at (a) Day 1, (b) Day 6, (c) Day 11, and (d) Day 16. Error bars represent 95% confidence interval for each data point (n = 6). Treatments were considered significantly different from each other when confidence intervals do not overlap at a given depth.
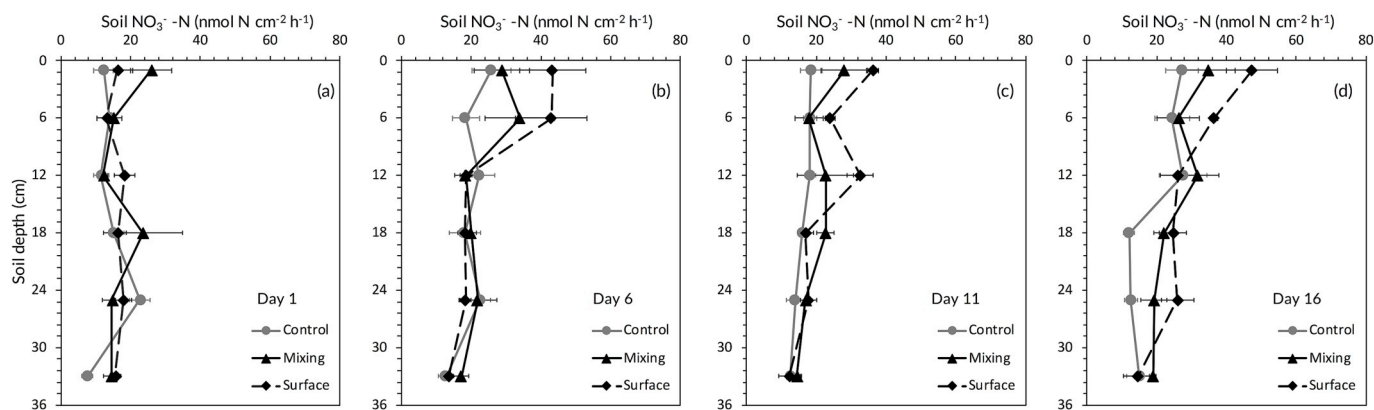
Fig. 3. Soil nitrate (NO_3^-) diffusive flux rates (nmol cm^{-2} h^{-1}) sampled by using microdialysis at varied depths in a soil column amended with no biochar (control), biochar mixed through the soil column (mixing), or surface addition of biochar (surface) in an incubation experiment at (a) Day 1, (b) Day 6, (c) Day 11, and (d) Day 16. Error bars represent 95% confidence interval for each data point (n = 6). Treatments were considered significantly different from each other when confidence intervals do not overlap at a given depth.

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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.2019.06.008.

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