Soil Biology and Biochemistry 165 (2022) 108541

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Root exudates shift how N mineralization and N fixation contribute to the plant-available N supply in low fertility soils

Yuan Liu [a,*], Sarah E. Evans [b], Maren L. Friesen [c], Lisa K. Tiemann [a]

[a] Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI, USA
[b] W.K. Kellogg Biological Station, Department of Integrative Biology, Michigan State University, Hickory Corners, MI, USA
[c] Departments of Plant Pathology and Crop and Soil Sciences, Washington State University, Pullman, WA, USA

ARTICLE INFO

Keywords:
Marginal lands
Root exudates
N fixation
Gross N mineralization
Plant-microbe interactions

ABSTRACT

Nitrogen (N) availability is a primary constraint to plant productivity, especially in marginal lands with inherently low fertility. Root exudates change with plant nutrient status, and are expected to affect the microbially-mediated N transformations (gross N mineralization vs N fixation) in low fertility soil (low soil organic matter). To explore this possibility, we sampled soils from two monoculture switchgrass (var. *Cave-In-Rock*) plot with and without N addition at two marginal land sites in Michigan, USA. In a two-week lab incubation, we quantified the effect of different root exudates on gross N mineralization and N fixation by adding simulated root exudates (carbohydrates, organic acids) at a rate of 100 μg C g^{-1} day^{-1}. On average, adding carbohydrates to low fertility soil increased the soil respiration by 254%, the dissolved organic carbon (DOC) by 366% and reduced dissolved organic N (DON) by 40%. In contrast, soils receiving organic acids had 159% more soil respiration, 163% higher DOC concentration and the DON concentration increased by 49%. Analysis of the C recovery in measured pools revealed that root exudates C inputs were nearly equivalent to the DOC, microbial biomass carbon (MBC), and soil respiration in sandy soil, but only 45–74% of the root exudate C was recovered in these pools in the sandy loam soil. This suggests that root exudate C may be adsorbed to mineral particles in the sandy loam soil. Soil treated with organic acids had higher gross N mineralization and N immobilization rates than soil with carbohydrates addition. Adding carbohydrates significantly increased the free-living N fixation rates, compared to organic acid addition. Changes in soil pH, and DON induced by root exudate addition had strong association with N transformation rates and N availability. Gross N mineralization produced more plant-available N than N fixation, as evidenced by higher inorganic N concentration in soils receiving organic acids than carbohydrates. By quantifying how different root exudates affect the contribution of N mineralization and N fixation to the plant-available N pool in low fertility soils, this study enhances our understanding of the "C for N" exchange in the plant rhizosphere.

1. Introduction

Nitrogen (N) mineralization and N fixation (free-living or symbiotic) are the two dominant processes controlling plant available N (Coskun et al., 2017); however, both are energy intensive processes (Smercina et al., 2019a). For example, N mineralization requires C for protease enzyme production, and N fixation requires large amounts of ATP energy to break the N_2 triple bond (Carnahan et al., 1960), which makes the energy or C supply for microbes an important controller of process rates (Roley et al., 2018). Plant root exudates, composed primarily of low molecular-weight organic compounds, including carbohydrates, organic acids, and amino acids (Dakora and Phillips, 2002), can be directly taken up into microbial cells, providing an ideal C source to support energy intensive N transformations. Therefore, root exudates have the potential to stimulate SOM decomposition in relation to N acquisition or alter N fixation rates and ultimately control soil N availability (Li et al., 2016; Meier et al., 2017). For example, Li et al. (2016) found maize root exudates increase nodulation and stimulate nitrogen fixation through enhanced gene expression, and Meier et al. (2017) found root exudates increase gross N mineralization in both fertilized and unfertilized soils. Despite the importance of the connection between root exudate C and processes controlling N availability, few studies have

* Corresponding author.
E-mail address: liuyua14@msu.edu (Y. Liu).

https://doi.org/10.1016/j.soilbio.2021.108541
Received 25 July 2021; Received in revised form 20 December 2021; Accepted 24 December 2021
Available online 28 December 2021

explored how root exudates impact N mineralization or N fixation and to our knowledge no study has simultaneously investigated the relative importance of N mineralization versus N fixation.

The amount and composition of root exudates is variable, depending on plant species (Bürgmann et al., 2005) and nutrient status (Smercina et al., 2020a). For example, previous work has demonstrated that under different N conditions, a perennial grass excretes different root exudate profiles; in N limited conditions organic acids were relatively more abundant than carbohydrates, while the opposite was true in non-N limited conditions (Smercina et al., 2020a). In general, carbohydrates-based metabolism is found more commonly in or is favored by a larger proportion of soil microorganism, while organic acids are metabolized by a smaller group of more specialized microorganisms (Bürgmann et al., 2005; Landi et al., 2006). However, organic acids were found to play a more important role than carbohydrates in shaping the structure of microbial communities within the rhizosphere (Landi et al., 2006; Shi et al., 2011). This might be due to shift in soil pH or solubilization of soil organic matter that affect the microbial community composition (Rousk et al., 2009). For example, Shi et al. (2011) found significant change in soil pH after organic acids addition compared with carbohydrates addition, which induced greater increases than sugars in the soil microbial community richness. Therefore, different root exudates might have different effects on N transformations through changes in both the structure and function of soil microbial communities. Although some studies have focused on how root exudates affect N mineralization or N fixation, results on how different root exudates affect these N transformation processes are not consistent. For example, Rao (1978) found evidence that addition of different organic acids (succinate, butyrate, pyruvate, and acetate) stimulated N fixation, while Bürgmann et al. (2005) showed that none of the organic acids treatments induced N fixation. Furthermore, previous studies separately investigate the effects of root exudates on N mineralization or N fixation (Meier et al., 2017; Smercina et al., 2019b), however, no studies have measured both N mineralization and N fixation simultaneously in response to different root exudates and assess the relative importance of N mineralization and N fixation in controlling the soil N availability.

In agroecosystems, N addition has been shown to be a major control on N transformations. N additions can either increase, decrease, or have no effect on soil gross N mineralization (Cheng et al., 2019), and N addition has also been shown to either decrease or not change N fixation rates. N addition can also alter N mineralization and N fixation by changing overall microbial community structure (Reed et al., 2010). More specifically, N addition can change the richness and abundance of N-fixing microbes (Freedman et al., 2013). For both N mineralization and N fixation responses to N addition have been mixed, but a better understanding of how N addition affects these N transformations will be necessary for successful and sustainable nutrient management. In bioenergy cropping systems, nutrient management with an eye towards sustainability is particularly important. Switchgrass (*Panicum virgatum* L.), a perennial, warm-season, C4 grass, has been well-studied for potential as a cellulosic biofuel (Rodrigues et al., 2017). N is the most commonly limiting nutrient for switchgrass, especially in marginal lands, which are unsuitable for food crops due to the inherently low fertility soils and vulnerability to environmental stress (Kang et al., 2013). A recent study found that switchgrass yields do not respond to increasing N additions once switchgrass has been established and that it will remain productive under very low N addition, even as N is being removed via annual harvest (Ruan et al., 2016). Understanding how switchgrass maintains its N demands, and where this 'missing' N is coming from in these low fertility, marginal lands, will allow us to optimize these bioenergy cropping systems to enhance their long-term sustainability.

To elucidate the effects of different root exudates on the relative importance of N mineralization and N fixation in the switchgrass rhizosphere, we sampled soils from experimental monoculture switchgrass split-plots with or without N addition, at two marginal land sites at the Great Lakes Bioenergy Research Center. During a two-week incubation, we added different root exudate cocktails with varying carbohydrates or organic acids concentrations into soils at a rate of 100 μg C g^{-1} soil per day. After incubation, we used ^{15}N incorporation and ^{15}N pool dilution methods to measure N fixation and gross N mineralization and immobilization rates. We hypothesized:1) both carbohydrates and organic acids addition would increase N fixation and gross N mineralization rates because these are two energy intensive processes; 2) organic acids would increase N fixation relatively more than N mineralization because organic acids play a more important role than sugars in shaping microbial community structure within the rhizosphere by recruiting more diazotrophs; and long-term N fertilizer additions would alter microbial communities, with decreases in N fixation and increasing gross N mineralization. Although this final hypothesis is supported by previous studies, current work at our study sites suggests there may actually not be a typical fertilizer response in these switchgrass systems (Smercina et al., 2021).

2. Materials and methods

2.1. Soil sampling

Soils were collected from two marginal land sites in Michigan, USA, Lux Arbor (LUX; 42.476365, −85.451887) and Lake City (LC; 44.296098, −85.199612). These field sites are maintained as part of the Great Lakes Bioenergy Research Center's Marginal Land Experiment (MLE; https://www.glbrc.org/). Soils at LUX are Typic Hapludalf loams (510 g sand kg^{-1}, 32 g silt kg^{-1}) with 7.7 g C kg^{-1} and 0.6 g N kg^{-1}. Soils at LC are Oxyaquic Haplorthod sandy loams (850 g sand kg^{-1}, 80 g silt kg^{-1}) with 9.2 g C kg^{-1} and 0.6 g N kg^{-1} (Kasmerchak and Schaetzl, 2018). Each field site has four replicate split plots of switchgrass (*Panicum virgatum* L. cv. Cave-in-Rock) monoculture which have been maintained since 2013. Split plots are divided in half, into two N addition levels (Nlevel), +56 kg urea-N ha^{-1} $yr^{-1}$and no added N. We sampled soils from both N levels to test the root exudates effect on soil N transformation processes with different N availability in different soils and climate conditions.

In October 2019, soils were collected using a PVC auger (5 cm in diameter) to a depth of 10 cm at least 1 m from the edges of switchgrass plots. Soils were collected close to (within 10 cm of the crown) a single switchgrass plant in order to generate soil samples completely under the influence of switchgrass roots. Three soil samples were collected from each split-plot and were combined to form a composite sample. Soils were kept in cooler until returning to the lab where they were stored at 5 °C until analysis. Visible roots and litter residues were manually removed from each soil sample and soils were sieved (2 mm mesh) and homogenized. Soil moisture was determined using 8 g of fresh soil dried at 60 °C for at least 24 h.

2.2. Artificial root exudate cocktail and soil incubation

To test the effect of root exudate excreted by switchgrass under different N scenarios, four root exudate cocktails were prepared with nano-pure water as outlined in Table 1. The composition and concentration of carbohydrate and organic acids in these solutions were based on previous work identifying switchgrass root exudate profiles under both high and low N conditions (Smercina et al., 2020a). 40 g dry mass equivalent soil was put into a 4 oz glass jar for each of the four split-plot field replicates from each site and for each root exudate treatment plus a water only control (n = 2 sites*4 blocks*2 split-plots*5 exudate or water treatments = 80 jars). Root exudate cocktails were applied daily to soils using a syringe and 21-gauge needle at a rate of 100 μg C g^{-1} dry soil. We applied exudate cocktails daily to better mimic the continuous secretion of root exudates in the field. Soils were incubated at 22 °C with jars open slightly every night to ventilate and dry in order to keep the moisture between 55 and 70% water holding capacity.

Table 1
Composition and rates of carbohydrates and organic acids in root exudate cocktails.

Treatment	Daily C input (μg C g^{-1} soil)	Ratio of Crb and OA	Carbohydrate (μg C g^{-1} soil)		Organic acid (μg C g-1 soil)	
			Arabinose	Fructose	Sodium Acetate	Succinic acid
W[1]	0	0: 0	/	/	/	/
Crb	100	100: 0	50	50	/	/
OA	100	0: 100	/	/	50	50
HCLOA	100	82: 18	41	41	9	9
LCHOA	100	30: 70	15	15	35	35

[1] W, control treatment with only nano-pure water; Crb, only carbohydrate; OA, only organic acid; HCLOA, high proportion of carbohydrate and low proportion of organic acid; LCHOA, low proportion of carbohydrate and high proportion of organic acid.

2.3. Soil respiration rates

Every day before adding the root exudate cocktails, the background soil respiration rate was monitored by a gas flow system attached to a LI-820 Infrared Gas Analyzer (LI-Cor Biosciences, Lincoln, NE, USA) by taking two gas samples 60–120 min apart from the headspace of the sealed jars. After adding the root exudate cocktails, CO_2 concentration in the headspace of the re-sealed jars was monitored by taking headspace gas samples at 0.5 h, 4 h, and 8 h after cocktail addition. Soil respiration rates between two measured time points were calculated based on linear interpolation. Cumulative CO_2–C respired each day, or the ~24 h that elapsed between root exudate additions, was calculated by multiplying the average daily respiration rate (average of rate measured before and after root exudate addition) by time between root exudate additions.

2.4. Nitrogen fixation rates

We measured N fixation rates based on the net changes in ^{15}N of soil incubated with $^{15}N_2$ gas (Smercina et al., 2019b). At the end of incubation, triple the daily rate of root exudates (300 μg C g^{-1} dry soil) were added to each soil in enough water to bring the soil to 65% water holding capacity. Then, a 10 g soil subsample was weighed into a 22 ml glass scintillation vial. Vials were then capped with aluminum rings and septa and evacuated to remove ambient atmosphere. After evacuation, vials immediately received 1 ml of 98 atom% acid-washed $^{15}N_2$ gas (Sigma-Aldrich, Inc., St. Louis, MO, USA) and reference vials received UHP-N_2. Following N_2 addition, 0.6 ml of oxygen was added to the vials to achieve a 5% oxygen headspace (Smercina et al., 2019a). Vial were brought to a final pressure of 1 atm with 10.4 ml UHP-He. Soils were incubated at room temperature for three days. After incubation, vials were uncapped, and samples were placed in a 60 °C drying oven for 48 h, then ground into a fine powder on a roller mill, weighed into tin capsules, and analyzed following standard procedures at Washington State University's Stable Isotope Core Laboratory (Pullman, WA). Briefly, tinned samples were combusted to N_2 with an ECS 4010 elemental analyzer (Costech Analytical, Valencia, CA) and analyzed on a Delta PlusXP continuous flow isotope ratio mass spectrometer (Thermo-Finnigan, Bremen, Germany) equipped with a 3 m GC column. Details can be found in Smercina et al. (2019a). N fixation rates were calculated as μg N fixed g^{-1} soil day $^{-1}$ using the equation:

$$\frac{AE_i * TN_i}{AE_{atm} * t}$$

where AE_i represents atom percent excess of sample against an unenriched reference sample, TN_i represents total nitrogen content in sample, AE_{atm} represents atom percent excess in the vial atmosphere (98 atom% in our case), and t is incubation time in days (Smercina et al., 2019b).

2.5. Gross N mineralization and N immobilization rates

Gross N mineralization and gross N immobilization rates were determined using the ^{15}N isotope pool dilution method as described by Hart et al. (1994). Briefly, 8 g soil subsamples were weighed into 50 ml polypropylene centrifuge tubes in triplicate. The first of the triplicate soil samples was then immediately extracted with 40 ml of 1 M KCl by placing on a shaker table at ~200 rpm for 30 min, centrifuging and finally filtering through Whatman #4 filter paper. These soil extracts were used to determine the amount of inorganic ^{15}N needed for the pool dilution. The remaining two of the triplicate soil subsamples had 0.5 ml of $(^{15}NH_4)_2SO_4$ (99% atom% ^{15}N, Cambridge Isotope Laboratories, MA, USA) added, bringing the final water content to 75% of WHC. The ^{15}N addition increased the ambient NH_4^+ pool size by about 2 mg of N kg^{-1} of dry soil (Hart et al., 1994). Labelled soils were mixed vigorously to distribute the tracer solution homogenously and then incubated at room temperature. These soil incubations were terminated after 6 h (t_1) and 30 h (t_2) by extraction with 1 M KCl as described above. Soil extract NH_4^+ contents were measured colorimetrically in 96-well plates as described by Sinsabaugh et al. (2000). 25 ml soil extract was pipetted into 90 ml polypropylene specimen cups. We added additional NH_4Cl to increase the total N concentration in each cup to 40.5 μg if necessary. The cups were capped immediately after addition 0.4 g MgO power and an acid trap. Each acid trap consisted of two 4 mm diameter Whatman quartz microfiber filter paper circles soaked with 10 μl of 2.5 M $KHSO_4$, that were wrapped in an 8 cm long strip of Teflon tape and sealed with the inverted cap of a micro-centrifuge tube. Each cup was gently swirled at least once per day for six days. After six days, the acidified quartz fiber filter paper was picked out from the Teflon tape and placed inside silver capsules and dried overnight at 60 °C. Isotope composition of the N on the acid trap discs was determined at the Washington State University's Stable Isotope Core Laboratory as described above. Gross N mineralization and N immobilization rates were calculated according to the equations described by Bengtson et al. (2005).

2.6. Soil pH, microbial biomass, dissolved organic C and N, ammonium and nitrate

Soil pH at the beginning and after incubation were measured using S20 SevenEasy pH meter (Mettler Toledo, OH, USA). At the end of the incubation, soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were analyzed using the chloroform fumigation-extraction method (Vance et al., 1987). Briefly, 40 ml of 0.5 M K_2SO_4 was added to 8 g dry mass equivalent soil, one unfumigated and one directly fumigated for 24 h with 2-ml of ethanol-free chloroform to lyse microbial cells and accumulate microbial C and N (Wepking et al., 2019). Soils plus K_2SO_4 were placed horizontally on an orbital shaker set at 250 rpm for 1 h. Total organic C and N in the resulting soil extracts was then determined using a Vario Select TOC/TN analyzer (Elementar, Ronkonkoma, NY). Microbial biomass was estimated as the difference between the quantity of C and N between the fumigated and un-fumigated samples. Microbial biomass C and N were calculated as fumigated extractable organic C (DOC) or total dissolved N (TDN) minus un-fumigated DOC and TDN, divided by an efficiency factor of 0.45 (Jenkinson et al., 2004). Dissolved organic N (DON) was calculated by subtracting total inorganic N from TDN. The inorganic N (ammonium and nitrate) was measured using colorimetric methods. Ammonium was measured in clear 96-well plates as described by Sinsabaugh et al. (2000). Nitrate analysis protocol was adapted from the Nitrate Elimination Co. Inc. (NECi) Method N07-0003 (http://www.nitrate.com/node/164). This method uses nitrate reductase (E.C. 1.7.1.1) to convert NO_3^- to NO_2^- so that standard colorimetric chemistry (sulfanilamide-NED) can be used to determine NO_2^- in clear 96-well plates as described by (Doane and Horwáth, 2003). Absorbance was subsequently

measured at 660 nm or 540 nm for ammonium and nitrate analysis respectively, on a spectrophotometric microplate reader (Synergy H1, BioTek, Winooski, VT).

2.7. Statistical analyses

Prior to statistical tests, all variables were visually checked using histograms, density, and Q-Q plots. Data was tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test). Variables that did not meet the assumption of parametric statistical tests (normality and homoscedasticity of errors) were log-transformed or square root-transformed to fulfill assumptions of normality and homogeneity where necessary. We tested for fixed effects of sampling site, root exudates treatment and their interaction using linear mixed effect models using the r package *nlme* to analyze the responses of soil variables (pH, DOC, DON, DOC: DON, MBC, MBN, MBC: MBN, inorganic N), total cumulative CO_2–C respired, percentage of root exudate C recovery and soil N transformation processes (gross N mineralization, gross N immobilization, N fixation). Replicate field blocks were treated as a random effect. Effects of root exudate treatment on average respiration rate on days 1, 8, 10, 15 were tested by one-way ANOVA. Statistical comparisons among different root exudate treatments were performed using a "Bonferroni" pairwise post-hoc test. Correlations between different soil properties and soil N transformation processes were examined with *corrplot* package in R. Linear mixed effect model results showed that there were significant sampling site effects on most of the variables (Table S1) but only a few variables were significantly affected by the field level N addition. Therefore, all the figures are presented by different sites and grouped with N addition treatment. Finally, we built a structural equation model (SEM) to evaluate the direct and indirect pathways that regulate N transformation processes and inorganic N, and to evaluate how these factors contribute to the standardized total effects (direct effects plus indirect effects). Predicted causal relationships between variables were based on prior knowledge of how different soil variables affect N transformation processes. The adequacy of the model was determined by the χ^2 -test, goodness of fit (GIF> 0.90) index, and root mean squared error of approximation (RMSEA <0.08) index (Liu et al., 2017). The SEM analysis was conducted using Amos 21.0 (Amos Development Corporation, Chicago, IL). All the statistical analyses, except SEM, were performed using R statistical software v3.2.4 (R Development Core Team, 2016). The significance of the analyses was set at $p < 0.05$ level. *ggplot2* package was used for plotting graphs (Wickham, 2016).

3. Results

3.1. Root exudate addition induced significant changes in soil pH and different C pools

Root exudate type significantly affected the soil pH (Table 2, Fig. S1). Overall, soil pH increased by 1–3 units with all treatments containing organic acids (OA) compared to control at both sites at the end of the incubation (Fig. 1a). In contrast, carbohydrate (Crb) treatment had no significant effect on soil pH at either site, and these changes in pH were not affected by N addition. Adding root exudates significantly increased soil respiration throughout the incubation (Fig. 1b; Figs. S2–3). On average, adding carbohydrates stimulated soil respiration to a greater extent than organic acids (Fig. 1b). Soil respiration in Crb and high carbohydrate and low organic acid (HCLOA) treatments were increased by 249% and 243% respectively, while OA and low carbohydrate and high organic acid (LCHOA) treatments increased by 154% and 188% respectively compared to control (Fig. 1b). Every day after root exudate addition, there was a respiration pulse that resulted in 2x greater respiration rates compared to just prior to root exudate additions, across all treatments and sites (Fig. S2). MBC increased significantly after Crb addition compared with control in Lake City under field N addition treatment. In contrast, no significant effect of root exudate type on MBC was found in Lux Arbor soil. Root exudate type had no significant effect on MBN at either site. However, N addition significantly increased MBN in Lake City, but significantly decreased MBN in Lux Arbor soils.

In Lake City soils, DOC was significantly greater across all root exudate types compared with control and OA was significantly greater than Crb and HCLOA (Fig. 2a). In Lux Arbor soils, there was a significant interaction between root exudate and N addition on DOC (Fig. 2a). With no N addition, all root exudate treatments had significantly increased DOC compared with control, with no differences among root exudate treatments; with N addition, all root exudate treatments but not Crb treatments, had significantly increased DOC compared with control (Fig. 2a). DON at Lake City was greatest in OA and LCHOA and lowest in Crb and HCLOA treatments (Fig. 2b) with no significant effects of N

Table 2
Summary (F-values) of two way-ANOVA for the effects of N addition (Nlevel), root exudate types (RE) and their interactions on soil properties and N transformation processes in two site.

Response variable/Site	Lake City			Lux Arbor		
	N addition (Nlevel)	Root exudate types (RE)	Nlevel × RE	Nlevel	RE	Nlevel × RE
pH_s	<0.001	3.58*	0.16	0.48	7.83***	0.02
pH_{end}	<0.001	128***	0.71	4.47***	187***	0.96
ΔpH	0.43	42.5***	1.54	0.23	52.6***	5.46***
MBC	1.95	4.30**	0.31	1.24	0.49	0.17
MBN	47.1***	2.89*	1.17	15.7***	0.12	0.82
MBC: MBN	2.16	2.96*	0.47	10.9**	0.68	0.27
DOC	0.23	24.5***	0.10	31.9***	27.9***	4.49**
DON	0.74	32.4***	0.19	24.1***	16.4***	0.68
DON: DON	0.03	9.19***	0.23	1.02	32.0***	2.25
Respiration rate	3.90	132***	0.15	13.2***	213***	0.27
Cumulative CO_2	3.90	132***	0.15	13.2***	213***	0.27
C recovery	0.89	0.82	0.25	4.92*	0.43	0.63
Inorganic N	2.65	0.28	0.66	<0.01	6.71**	0.95
$GrossN_{min}$	2.99	1.73	1.02	4.62*	13.9***	0.29
$GrossN_{immob}$	2.15	1.44	1.11	4.97*	12.6***	0.39
$NetN_{min}$	5.87*	1.01	1.77	0.21	1.26	1.26
N fixation	4.23*	7.20***	0.57	2.87	15.7***	7.23***
N_{min}: N_{fix}	1.07	8.54***	2.39	1.36	23.7***	1.11

Abbreviations: ΔpH was calculated by subtracting the initial soil pH (pH_s) from soil pH after incubation (pH_{end}); MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MBC: MBN, the ratio of MBC and MBN; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; DOC: DON, the ratio of DOC and DON; $GrossN_{min}$, gross N mineralization; $GrossN_{immob}$, gross N immobilization; $NetN_{min}$, net N mineralization; N_{min}: N_{fix}, the ratio of gross N mineralization and N fixation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

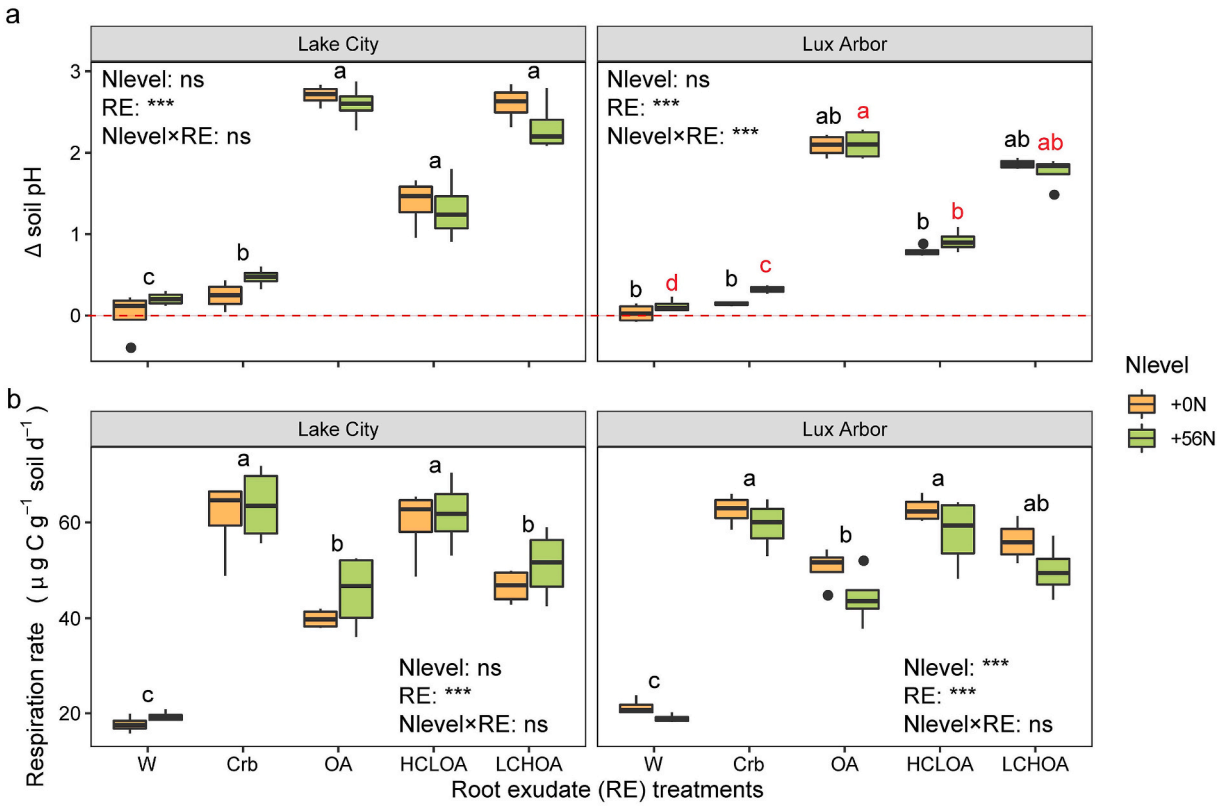

Fig. 1. Changes in soil pH (ΔpH = difference in pH before and after incubation) and average soil respiration rate among different root exudates and N addition levels. Values are average (±SE) of four field replicates. The red dashed line at zero indicates no change in pH. The effects of N addition (Nlevel) and root exudate types (RE) and their interaction were tested by using two-way ANOVA; ns represent non-significant difference, *, **, *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Different lower-case letters represent significant differences among root exudate (RE) treatments, with different color letters representing differences by N addition level: +0N (black); +56N (red). W: nano-pure water control; Crb: carbohydrate treatment; OA: organic acid treatment; HCLOA: high fraction of Crb and low fraction of OA treatment; LCHOA: low fraction of Crb and high fraction of OA treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

addition. In Lux Arbor soils, Crb and HCLOA treatments decreased DON compared with OA and overall, DON was significantly reduced with N addition. The ratio of DOC:DON was significantly widened by all root exudate additions compared to control at both sites (Fig. 2c). There were significantly different C recovery patterns between the two sites (Fig. 3; Table S1, $p < 0.001$). In Lake City soils, the total C recovered in DOC, MBC, and respired C pools accounted for 92%–106% of the total added root exudate C with no significant differences in total C recovered between root exudate treatments or N addition levels (Fig. 3; Table S1). In contrast, in Lux Arbor soils, only 45%–74% of the added root exudate C was recovered in these same three C pools, with no significant differences in recovery between different root exudate treatments, while the total C recovered was significantly higher without N addition compared to N addition (Table 2).

3.2. Root exudates shift how N mineralization and N fixation contribute to plant-available N

There is a strong correlation between gross N mineralization and gross N immobilization (Fig. S5). In Lake City soils, overall, there were no significant root exudate type or N addition effects on either gross N mineralization or gross N immobilization (Fig. 4ab). In Lux Arbor soils, Gross N mineralization and gross N immobilization were significantly increased with OA compared with Crb and HCLOA treatments (Fig. 4ab), and both gross N mineralization and gross N immobilization were reduced with N addition. No significant root exudate type or N addition effects were found on net N mineralization at either site (Fig. 4c). N addition and root exudate type had varied effects on N fixation rates (Table 2; Fig. 5). In Lake City soils, N fixation were lowest in OA compared to all other root exudate types (Fig. 5). In Lux Arbor soils, there was a significant interaction between root exudate type and N addition such that soils with no N addition (+0N) had the highest N fixation with Crb addition. In Lux Arbor soils receiving N (+56N), N fixation was higher only in HCLOA compared to OA. The ratio of gross N mineralization and N fixation (N_{min}: N_{fix}) was not significantly affected by N addition at either site but did vary by root exudate type (Fig. 6a; Table 2). In both sites, the N_{min}: N_{fix} ratio was wider after OA compared to Crb and HCLOA additions. In Lake City, inorganic N was not significantly affected by either root exudate type or N addition (Table 2). In contrast, root exudate type had a significant effect on inorganic N in Lux Arbor soils (Fig. 6b), with greater inorganic N with OA addition compared with Crb and HCLOA root exudate types.

3.3. Root exudate induced pH and DON change had strong correlation with N transformations and soil N availability

Correlation analysis showed that gross N mineralization or gross N immobilization were significantly positively correlated with soil pH at the end of incubation (pH_{end}), DOC, DON, and inorganic N, but negatively correlated with soil respiration (Fig. S6). N fixation was negatively affected by increasing pH_{end} and DON and positively correlated with soil respiration. N_{min}: N_{fix} was positively correlated with DOC, DON, and soil pH_{end}, and negatively correlated with soil respiration rate. Net N mineralization was only positively correlated with DON. In addition, inorganic N had positive correlations with soil pH_{end}, DOC, and DON (Fig. S6). Changes in soil pH induced by root exudate additions and

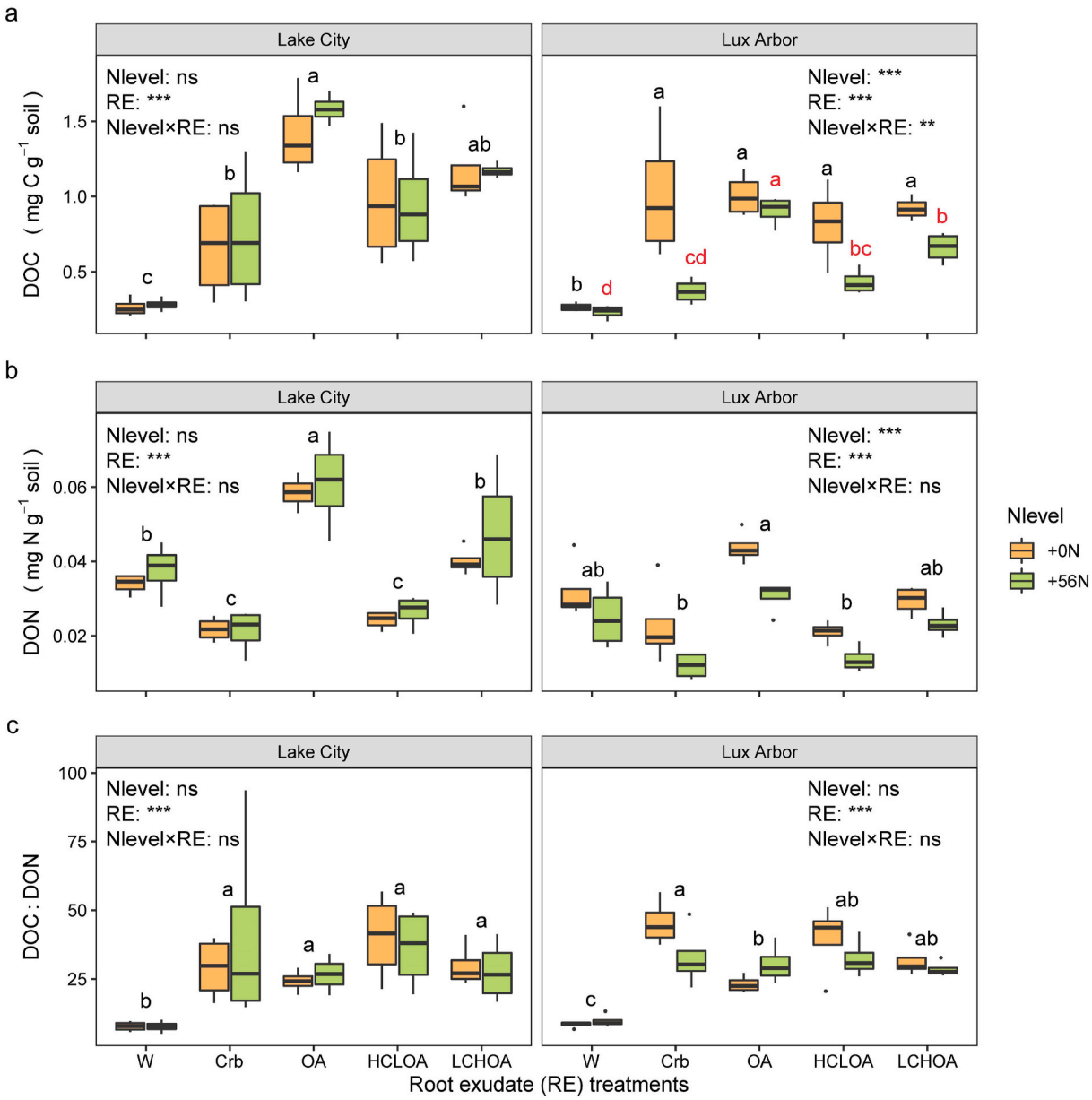

Fig. 2. Dissolved organic carbon (DOC, a) dissolved organic nitrogen (DON, b), and the ratio of DOC: DON (c) among different root exudate types and N addition levels in Lake City and Lux Arbor soils. Values are average (±SE) of four field replicates. The effects of N addition (Nlevel) and root exudate types (RE) and their interaction were tested by using two-way ANOVA; ns represent non-significant difference, *, **, *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Different lower-case letters represent significant differences among root exudate (RE) treatments, with different color letters representing differences by N addition level: +0N (black); +56N (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

subsequent changes of dissolved organic C and N (DOC, DON) were the main influencing factors in controlling the different N transformation processes and soil inorganic N availability (Fig. 7). Gross N mineralization was mainly associated with soil pH_{end} and DON, while N fixation was indirectly associated by DOC and directly correlated with microbial activity (soil respiration). Finally, gross N mineralization was more important than N fixation in controlling inorganic N availability in these marginal land soils.

4. Discussion

4.1. Root exudates shift the relative contribution of N mineralization and N fixation to N availability

Soil N availability is determined by the balance of N mineralization and N fixation. However, previous studies solely focus on how root exudates affect either N mineralization or N fixation processes (Bürgmann et al., 2005; Le Roux et al., 2008; Meier et al., 2017). How root exudates (carbohydrates *versus* organic acids) affect the relative importance of N mineralization and N fixation is unknown. This study, to the best of our knowledge, is the first to quantify how different root exudates affect the relative importance of N mineralization and N fixation in controlling soil N availability (Fig. 8). Because we lack gross N mineralization measurements with water only addition, we cannot test the hypothesis that all root exudate additions increase N mineralization rates, but we found higher gross N mineralization after adding organic acids compared with carbohydrates. We also found a significant increase in DON after adding organic acids (Fig. 2b), which might be due to dissociation of mineral-associated OM. The increase of soil pH with OA addition, which we discuss below (4.2), is evidence for dissociation of mineral-associated

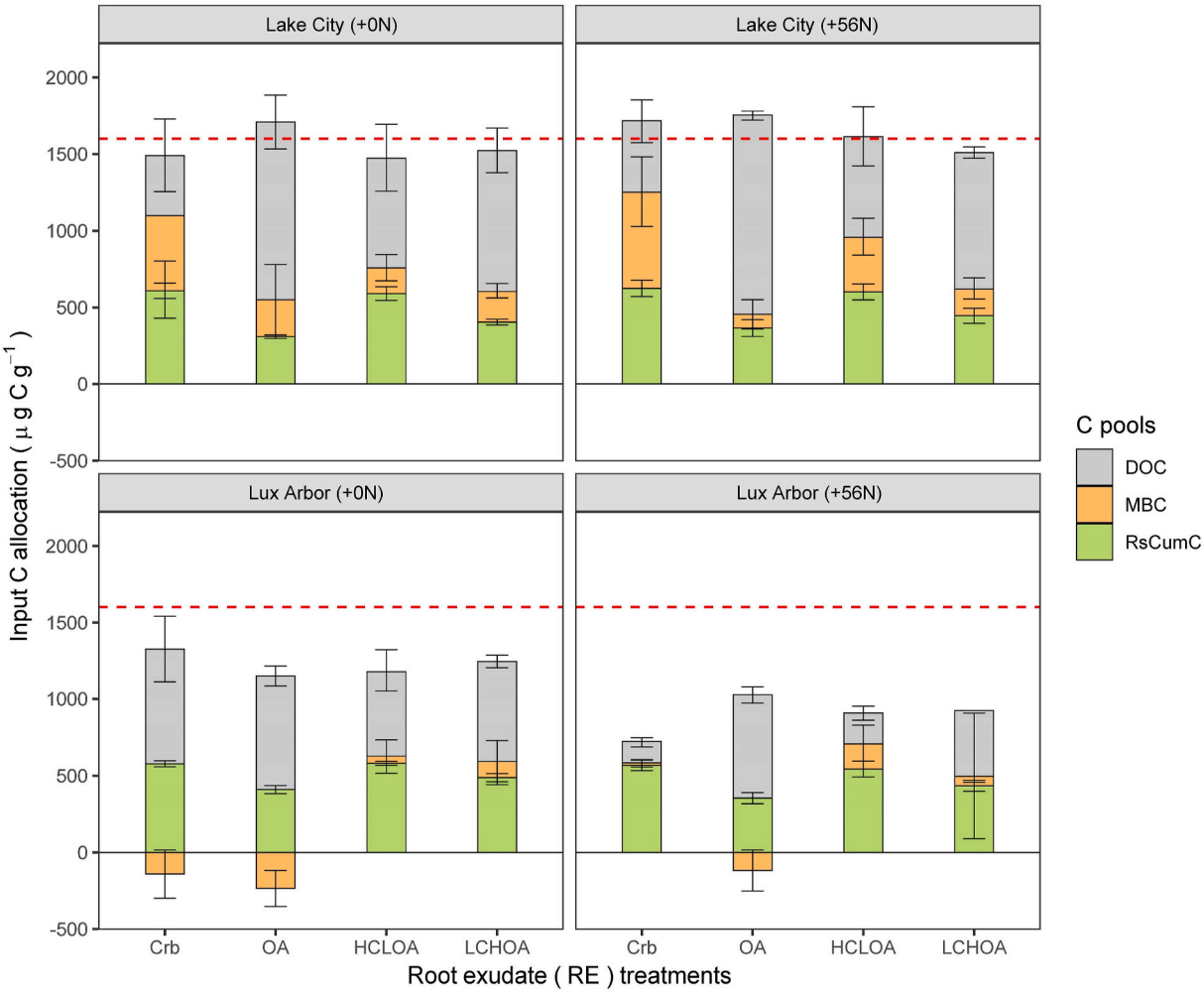

Fig. 3. Measured C pools after incubation with root exudate addition relative to the water only treatment in Lake City and Lux Arbor soils receiving two levels of N addition 0 and 56 kg N ha^{-1}. C pools include dissolved organic carbon (DOC), microbial biomass carbon (MBC) and cumulative C respired (RsCumC). The red dashed line indicates the total amount of root exudate C added over the two-week incubation. Note: negative MBC means a decrease of MBC in the root exudate treatment relative to the water control. Although partitioning between C pools differed, there were no significant differences in total C recovered across root exudate (RE) treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

OM. In any case, the increase in DON after organic acids addition compared with carbohydrates, provided more substrate for N mineralization.

Adding carbohydrates significantly increased soil N fixation, which is in agreement with previous studies (Rao, 1978; Bürgmann et al., 2005; Li et al., 2016) and partly supports our first hypothesis that all root exudate additions will increase N fixation. This is not surprising because N fixation is an energy intensive process, and carbohydrates used in this study are compounds that can be directly taken up into microbial cells without release of additional extracellular enzymes and will yield relatively high amounts of energy to support N fixation (Shi et al., 2011). For example, Rao (1978) previously showed that addition of glucose and sucrose stimulated N fixation. Similarly, Bürgmann et al. (2005) found that all sugar treatments significantly increased acetylene reduction, which is used as an indicator of increased nitrogenase activity and N fixation. Additionally, they also found the fastest increase and highest rates of acetylene reduction in treatments with glucose compared to fructose and sucrose, which suggests that N fixing microorganisms have different carbohydrate preferences. In contrast to our hypothesis, organic acids addition did not increase N fixation, but induced a slight decrease of N fixation, which is surprising because organic acid is also an ideal C source for the higher energy intensive N fixation. The only reason that there was no response of N fixation to organic acid addition is that N fixers is not preferring the organic acid that we added. Acetate acid and succinic acid were the two abundant organic acid that were excreted by switchgrass under N limiting condition (Smercina et al., 2020a), which we presumably thought to recruit N fixers around the rhizosphere to increase the available N for the plant. A previous study also showed that none of the added organic acid induced N fixation (Bürgmann et al., 2005), which is also unexpected because that both citric and malic acid were consumed, and nitrate was depleted in a similar pattern as compared with sugar-containing treatments. Additionally, organic acid accumulation (high level of malate) under phosphorus deficiency condition were also found to associate with reduced N fixation (Le Roux et al., 2008). In contrast, Rao (1978) previously showed that addition of several organic acids (succinate, butyrate, pyruvate, propanol, acetate) stimulated N fixation, however, their experiment is under anaerobic condition, which is not comparable with our study that was conducted under aerobic condition. Overall, our results combined with previous studies suggested that N fixers have a preference use of carbohydrates over organic acids.

We found a significant increase of the ratio of gross N mineralization to N fixation (N_{min}: N_{fix}) after adding organic acids compared to carbohydrates, regardless of the concentration of organic acids (Fig. 8), and therefore a higher N availability in organic acids treatment than in carbohydrates treatment. This result is in contrast with our second

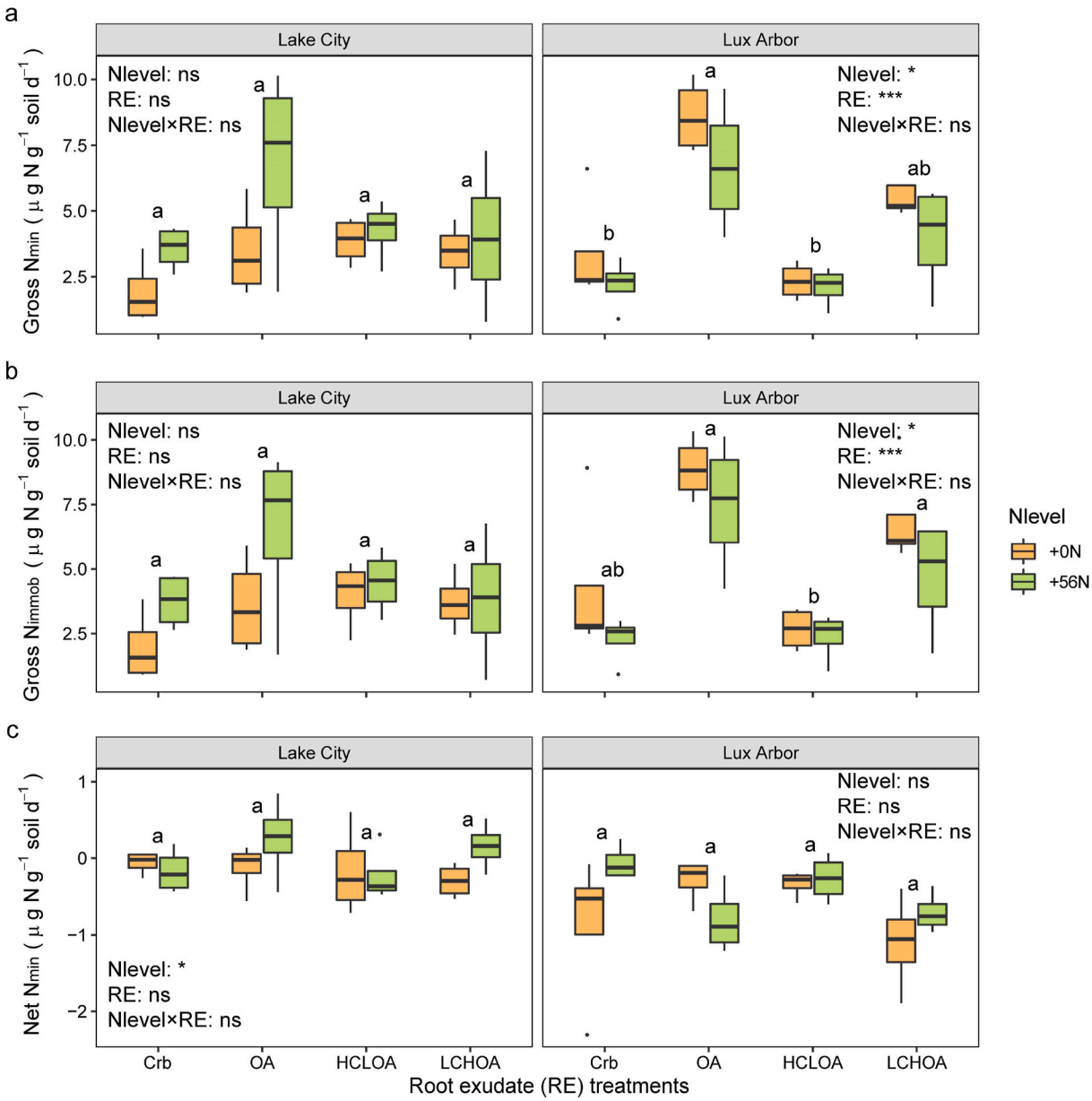

Fig. 4. Gross N mineralization (N_{min}), gross N immobilization (N_{immob}), and net N mineralization (NetNmin) among different root exudate types and N addition levels in Lake City and Lux Arbor. Values are average (±SE) of four field replicates. The effects of N addition (Nlevel) and root exudate types (RE) and their interaction were tested by using two-way ANOVA; ns represent non-significant difference, *, **, *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Different lower-case letters represent significant differences among root exudate (RE) treatments, with different color letters representing differences by N addition level: +0N (black); +56N (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

hypothesis that organic acids will increase N fixation more than N mineralization because organic acids are usually used by specialized microorganisms under N limiting conditions, which are throught to recruit N fixers (Smercina et al., 2020a). For example, Shi et al. (2011) found significant increase of N fixers (*Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Sphingomonadales* etc.) with root exudate cocktails with organic acids rather than with sugar additions. However, one of the organic acids used in our study, acetate acid, excreted under low N conditions by switchgrass, has been shown to completely inhibit nitrogenase activity of diazotrophs in pure cultures (Dobereiner et al., 1975). In addition, we found direct evidence that there was more DOC left (not respired) in the organic acid treatment than in the carbohydrate treatment after the incubation, which suggests that microorganisms in these soils may prefer carbohydrates as a C source over organic acids. This evidence can help to explain why the organic acids addition did not increase N fixation, and is in line with previous opinions that carbohydrates are favored by a larger proportion of soil microorganism, while organic acids are preferred by specialized microorganisms (Landi et al., 2006). Therefore, the secretion of organic acids by switchgrass might not be used for supporting N fixation, instead being used for stimulating N mineralization by destabilizing previously protected soil organic matter. Overall, our results suggest that N mineralization may play a more important role in controlling N availability than N fixation after addition of organic acids, while N fixation maybe more important in providing N to switchgrass when they excrete more carbohydrates in these marginal land soils.

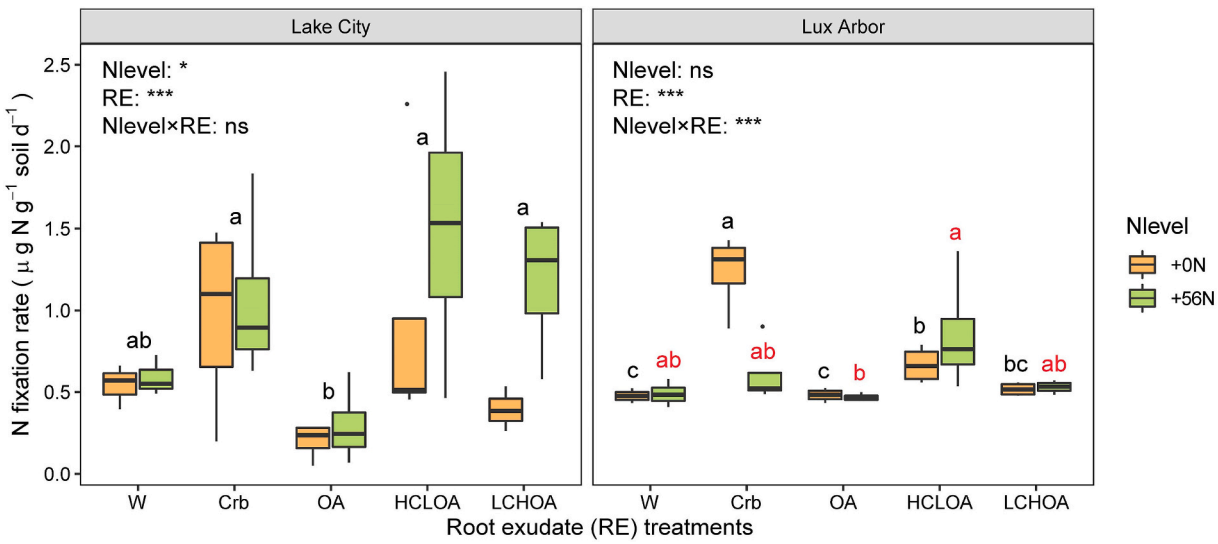

Fig. 5. Effects of different root exudate types and N addition levels on N fixation rates in Lake City and Lux Arbor soils. Values are average (±SE) of four field replicates. The effects of N addition (Nlevel) and root exudate types (RE) and their interaction were tested using two-way ANOVA; ns represents non-significant difference, *, **, *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Different lower-case letters represent significant differences among root exudate (RE) treatments, with different color letters representing differences by N addition level: +0N (black); +56N (red). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

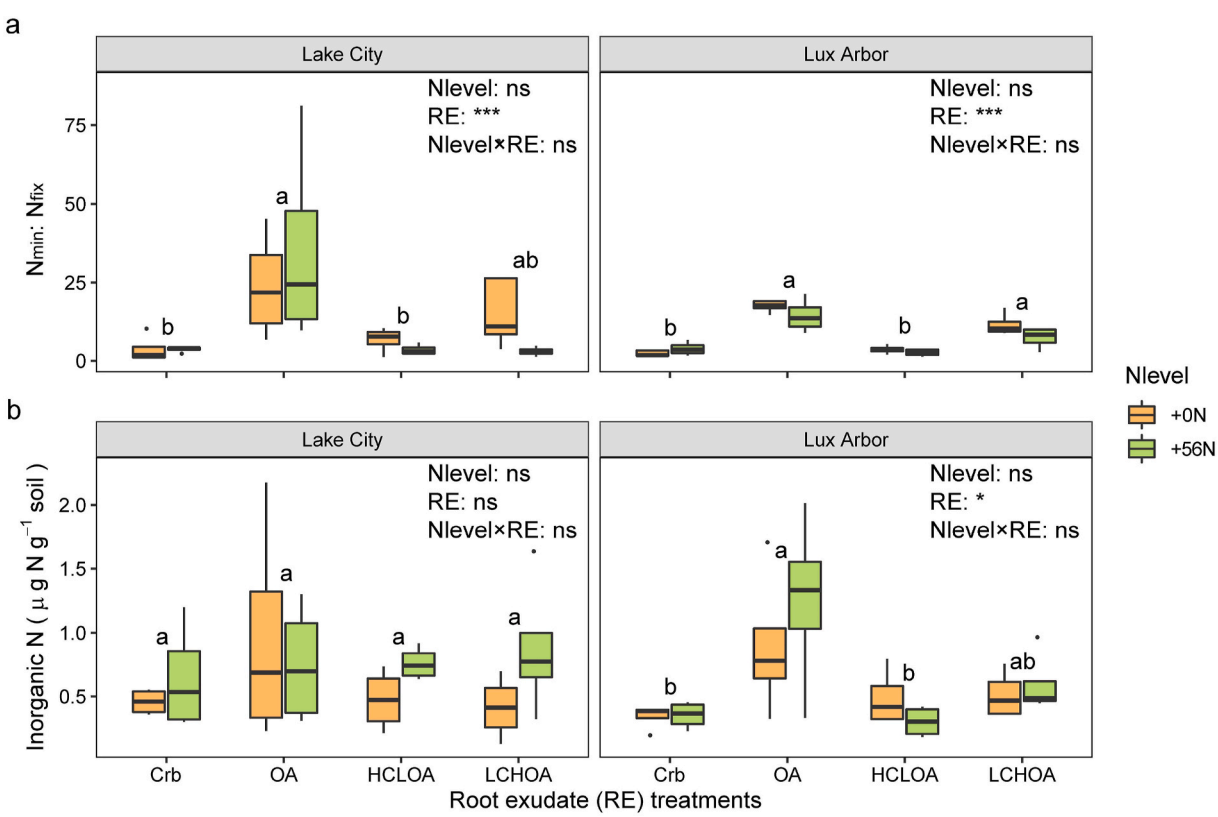

Fig. 6. The ratio of gross N mineralization and N fixation (N_{min}: N_{fix}) (a), and inorganic N (b) among different root exudate types and N addition levels in Lake City and Lux Arbor soils. Values are average (±SE) of four field replicates. The effects of N addition (Nlevel) and root exudate types (RE) and their interaction were tested using two-way ANOVA; ns represent non-significant difference, *, **, *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Different lower-case letters represent significant differences among root exudate (RE) treatments.

4.2. Root exudates mediate N transformations and N availability through different mechanisms in low fertility soils

We found that root exudates induced changes in soil pH and subsequent changes of DON and soil respiration had strong associations with different N transformation processes (Fig. 7). The pH-induced increase in N mineralization can be explained by the dissociation of the mineral-associated OM followed by increases in solubility of OM in soils with increased pH (Keiluweit et al., 2015; Curtin et al., 2016; Jilling et al., 2018). This is supported by previous studies that reported gross N

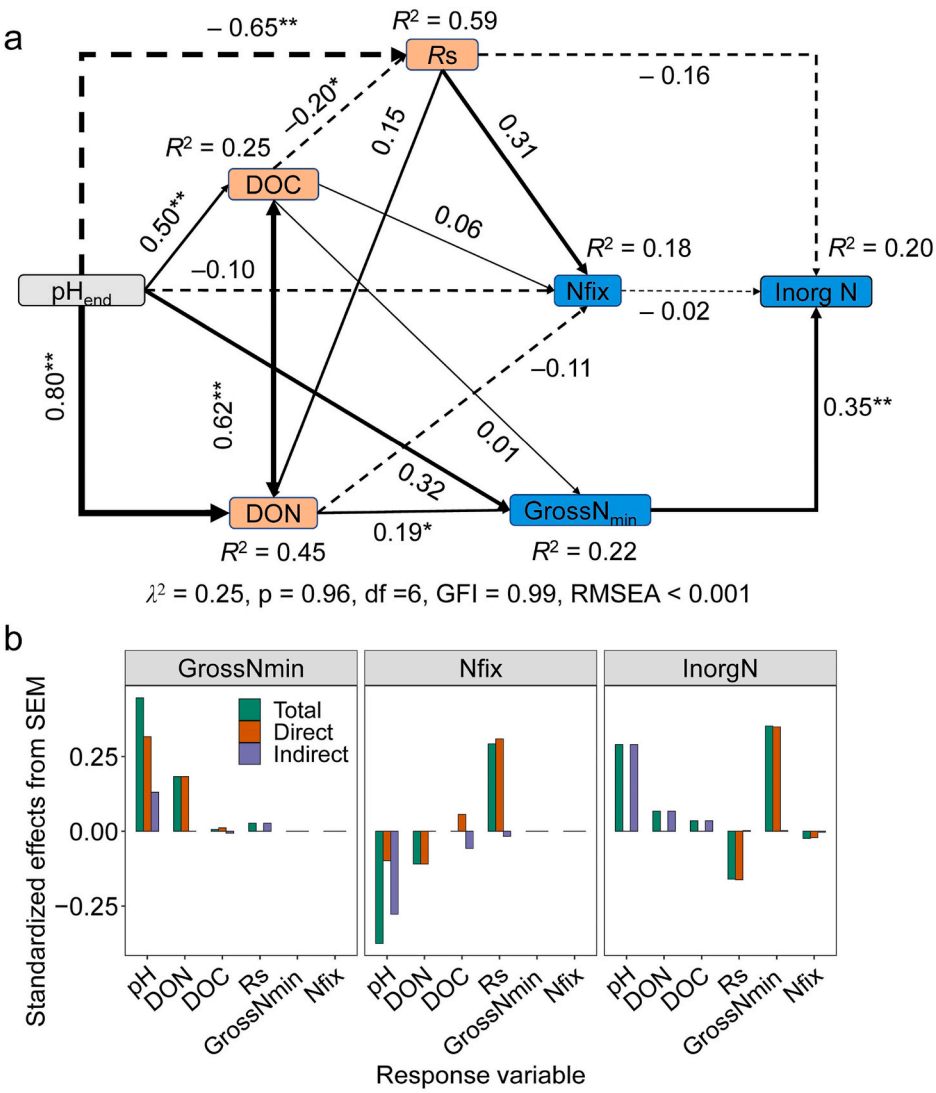

Fig. 7. Structural equation model (SEM) shows how root exudates affect N cycling. (a) solid lines represent positive relationships, and dashed line represent negative relationships; R^2 values associated with response variables indicate the proportion of variation explained by relationships with other variables. Numbers adjacent to arrows are standardized path coefficients (covariation coefficients) proportional to thickness of the lines, Significance: *, **, and *** represent $p < 0.05$, $p < 0.01$, and $p < 0.001$ level. pH_{end}, soil pH at the end of the incubation; DOC: dissolved organic carbon; DON, dissolved organic nitrogen; *Rs*: soil respiration rate; InorgN: inorganic N; $GrossN_{min}$, gross N mineralization; N_{fix}, N fixation. (b) SEM standardized total, direct, and indirect effects of different soil properties on $GrossN_{min}$, N_{fix}, as well as InorgN.

mineralization increased with increases of soil pH (Cheng et al., 2013). In our study, we found a significant increase of soil pH after adding organic acids, regardless of organic acids concentration, while carbohydrates did not change the soil pH (Fig. 1). This result is unexpected, because usually the secretion of organic acids in the plant rhizosphere will decrease the soil pH due to its acidity (Hinsinger et al., 2003). The increase of soil pH after adding organic acids can be explained by the degradation of carboxylic acids by soil microbes which consumes H^+ and liberates OH^- and CO_2 (Gramss et al., 2003). Furthermore, the acetate that we used in root exudate cocktails, and is commonly found in the rhizosphere, will also directly lead to increases of soil pH to some extent (Yu and Huang, 2009). This result is also consistent with several previous studies (Shi et al., 2011; Keiluweit et al., 2015; Girkin et al., 2018a,b), which also found organic acids addition increased the soil pH in lab incubation studies. For example, Shi et al. (2011) found a 1–3 unit increase of soil pH after adding a root exudates cocktail containing organic acids, which is comparable with our results.

In our study, the increases in pH were accompanied by increased DON (Fig. 2b), which provides more available organic substrate for N mineralization. Keiluweit et al. (2015) also provides direct evidence that organic acids (acetic acid) addition significantly reduces the amount of Fe and Al in metal-organic complexes and disrupts mineral-organic associations. Additionally, soil pH increase can enhance N mineralization by increasing the activity of soil enzymes involved in the N cycle (Vázquez et al., 2019). Furthermore, at higher pH, the molecular formulae of dissolved organic matter can have a lower degree of unsaturation and oxygenation, lower molecular size, and higher abundance of N-containing compounds available for higher biological decomposition activity (Roth et al., 2015).

The additional, and more biologically accessible dissolved organic matter (DOC more specifically in this case) also had a positive impact on N fixation as can be seen by the strong relationship between soil respiration and N fixation (Fig. 7). N fixation is an extremely expensive process energetically, requiring 16 ATP to break apart one di-nitrogen molecule (Smercina et al., 2019a). Therefore, respiratory costs for N fixation are high and are reflected in the relatively higher respiration rates associated with N fixation that are fueled by increased DOC availability. Overall, pH changes and subsequent increases in the bioavailability of dissolved organic matter (both DON and DOC) that were induced by different root exudate additions altered the relative importance of N mineralization and N fixation and as a result affected soil inorganic N availability in those low fertility soils.

4.3. C recovery patterns of added RE in marginal land soils

After two weeks incubation, we found different C recovery patterns between the two marginal land sites (Fig. 3). In Lake City soils, when MBC, DOC, and cumulative C respired were summed, we were able to

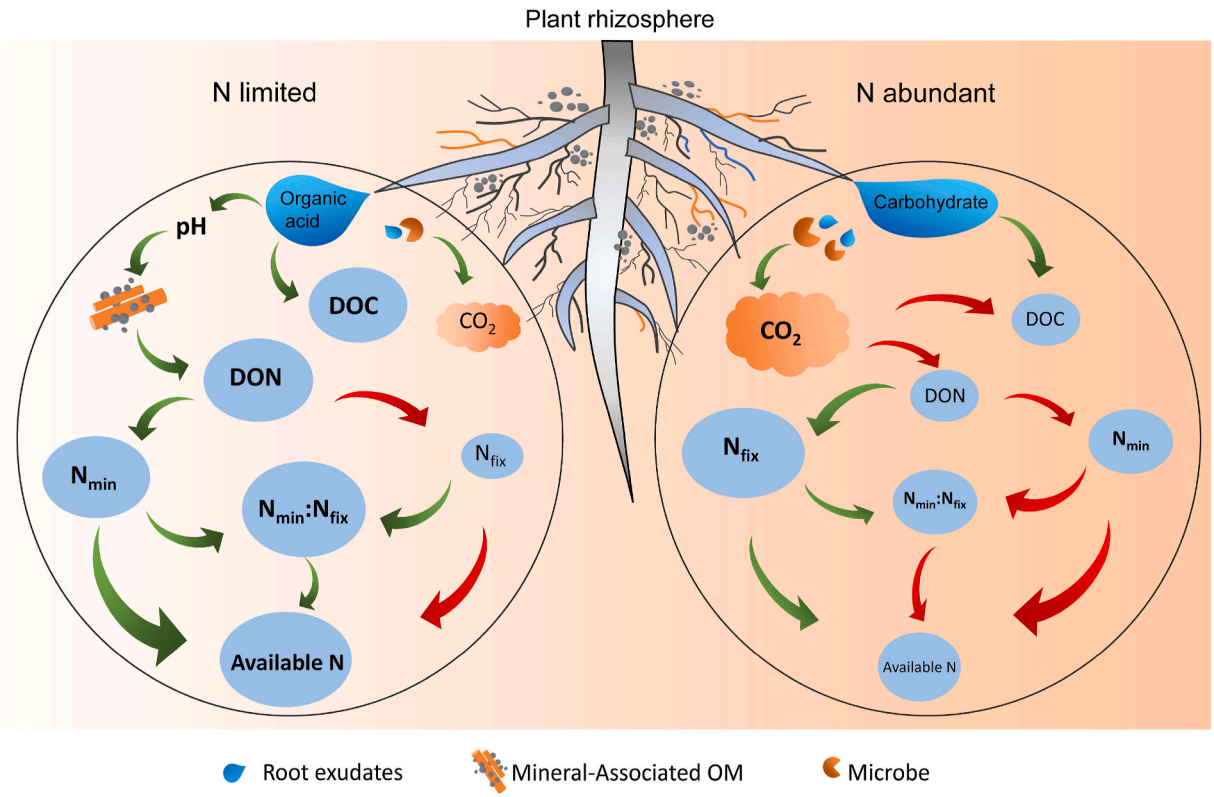

Fig. 8. Conceptual diagram showed potential mechanisms of root exudates (organic acid *versus* carbohydrate) on different N transformation processes in plant rhizosphere under different N condition. The circle size represents the magnitude of different pool or the flow in organic acid or carbohydrate treatment. Red arrows indicate negative effect, while green arrows indicate positive effect. The width of the arrows showed relative strength of different processes. The bolded and enlarged text (e.g., DOC) under N limiting condition on the left shows their higher increase with organic acid addition compared with carbohydrate addition, with a similar situation for text on the right without N limitation. DOC: dissolved organic carbon; DON, dissolved organic nitrogen; N_{min}, gross N mineralization; N_{fix}, N fixation; N_{min}: N_{fix}, the ratio of gross N mineralization and N fixation; N availability represented by inorganic N; Note that plant excrete more organic acid under N limiting conditions, whereas more carbohydrate without N limitation (Smercina et al., 2020a). Compared with carbohydrate addition, organic acid significantly increased soil pH, which induced release of mineral-associated OM and therefore, increased DON, that provide the substrate for N mineralization and increased the N_{min}, but the increased DON downregulate the N fixation, overall, N_{min} outcompete N fixation in regulating the availabilble N and increase the N availability under N limited condition. While carbohydrate addition significantly increased soil respiration, which depleted DOC and DON, the decrease DON therefore decrease the N_{min} but increase the N fixation. However, the influence of DON on N_{min} is high than N fixation, which decreased the final N availability under N abundant condition. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

account for 92–106% of the C we added as root exudates. In contrast, we found the recovered C in Lux Arbor soils only accounted for 45–74% of the total root exudate added C (Fig. 3). The potential mechanism explaining the low C recovery in these soils are that 1) the root exudate C was directly adsorbed onto mineral surfaces (Curtin et al., 2016; Yu et al., 2017) or 2) a faster microbial biomass turnover rate in the Lux Arbor soilswould lead to greater SOC through microbial necromass stabilization (Sokol and Bradford, 2019). Earlier work by Carter (1978) showed that DOC could be directly adsorbed onto $CaCO_3$. In our study, we found that carbonate content is higher in Lux Arbor (2.4%) compared to Lake City (1%) soils (data not shown). The high proportion of carbonate in Lux Arbor may have led to adsorption of some of the added root exudate C and partially explain the lower C recovery in these soils. In addition, there is more silt and clay and less sand in Lux Arbor compared to Lake City soils (Kasmerchak and Schaetzl, 2018). Sandier soils are often more limited in their capacity to build soil organic matter due to a limited capacity to protect C long-term by various mechanisms such as direct mineral surface interactions (Plante et al., 2006; Jilling et al., 2020). Therefore, the difference in soil texture may explain part of the low recovery of root exudate C in Lux Arbor as more clay surfaces available in these soils would also lead to greater potential for adsorption.

4.4. N addition have no significant effects on N transformations

Field level N additions had no significant effects on either N mineralization or N fixation rates without adding any root exudate, which is surprising, as we hypothesized that N addition would decrease N fixation and increase N mineralization. N additions have been shown to be a major control on N transformations, but no change in N fixation or N mineralization with N addition in our study is in contrast with previous studies (Cheng et al., 2019; Fan et al., 2019). These surprising results may be explained by the following reasons: first, the amount of N added in the field was relatively small at a dose of 56 kg N ha^{-1} $year^{-1}$, which is equal to the amount of N that is removed, on average, through annual switchgrass biomass harvests. This is a pretty low amount of N compared with other studies where N additions are as high as 140 kg N ha^{-1} $year^{-1}$ (Cheng et al., 2019). Also, N addition effects may disappear or attenuate to a negligible extent by the time we sampled the soil in October as the N was added as urea in May. Indeed, in a recent study at the same sites, N fixation rates were significantly different between N addition and no N addition treatments immediately after N addition, with differences disappearing for the remainder of the growing season (Smercina et al., 2021). In addition, previous results at the same site have shown there are few impacts of N addition on the microbial community (metagenome, unpub. results) including diazotrophs (Smercina et al., 2020b, 2021). In conclusion, the relatively low amount of N added, and

disappearance of N affects over time resulted in no response of N transformation processes to N fertilizer additions in these switchgrass system soils.

To summarize, this is the first study to quantify how different root exudates affect the relative importance of N mineralization and N fixation in marginal land soils. We showed that root exudates addition induced changes in pH and DON that in turn shift how N mineralization and N fixation contribute to plant available N in low fertility soils (Fig. 8). N mineralization appears to play a more important role than N fixation in regulating soil N availability. In addition, we found contrasting root exudate C retention in different soils, which were presumably caused by different soil texture and mineralogy. Future studies should also explore whether different N transformation processes will respond similarly to different types of organic acids or carbohydrates. It is also worthwhile to investigate the underlying mechanisms of how different soil textures and mineralogy affect root exudate C sorption and desorption processes and contributions to soil C accumulation. By quantifying how different root exudates affect the contribution of N mineralization and N fixation to the plant-available N pool in low fertility soils, this study enhances our understanding of the "C for N" exchange in the plant rhizosphere, which will be useful for reaching long-term sustainability goals in cropping systems.

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.

Acknowledgments

We would like to thank all members of the Tiemann lab who helped to collect and process samples. We thank Carolina Cordova from Phil Robertson's lab for coordinating the soil sampling. This work was conducted under the MMPRNT project, funded by the Department of Energy (DOE) Biological and Environmental Research (BER), Office of Science award DE-SC0014108. This work was also funded in part by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-SC0018409 and DE-FC02-07ER64494), by the National Science Foundation Long-Term Ecological Research Program (DEB 1832042) at the Kellogg Biological Station.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2021.108541.

References

Bengtson, P., Falkengren-Grerup, U., Bengtsson, G., 2005. Relieving substrate limitation-soil moisture and temperature determine gross N transformation rates. Oikos 111, 81–90.
Bürgmann, H., Meier, S., Bunge, M., Widmer, F., Zeyer, J., 2005. Effects of model root exudates on structure and activity of a soil diazotroph community. Environmental Microbiology 7, 1711–1724.
Carnahan, J.E., Mortenson, L.E., Mower, H.F., Castle, J.E., 1960. Nitrogen fixation in cell-free extracts of Clostridium pasteurianum. Biochimica et Biophysica Acta 44, 520–535.
Carter, P.W., 1978. Adsorption of amino acid-containing organic matter by calcite and quartz. Geochimica et Cosmochimica Acta 42, 1239–1242.
Cheng, Y., Wang, J., Chang, S., Cai, Z., Müller, C., Zhang, J., 2019. Nitrogen deposition affects both net and gross soil nitrogen transformations in forest ecosystems: a review. Environmental Pollution 244, 608–616.
Cheng, Y., Wang, J., Mary, B., Zhang, J., Cai, Z., Chang, S., 2013. Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in central Alberta, Canada. Soil Biology and Biochemistry 57, 848–857.
Coskun, D., Britto, D.T., Shi, W., Kronzucker, H.J., 2017. How plant root exudates shape the nitrogen cycle. Trends in Plant Science 22, 661–673.
Curtin, D., Peterson, M.E., Anderson, C.R., 2016. pH-dependence of organic matter solubility: base type effects on dissolved organic C, N, P, and S in soils with contrasting mineralogy. Geoderma 271, 161–172.
Dakora, F.D., Phillips, D.A., 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil 245, 35–47.
Doane, T.A., Horwáth, W.R., 2003. Spectrophotometric determination of nitrate with a single reagent. Analytical Letters 36, 2713–2722.
Dobereiner, J., Day, J.M., 1975. Nitrogen fixation in the rhizosphere of tropical grasses. In: Nitrogen Fixation by Free-Living Microorganisms. Cambridge University Press, London, United Kingdom.
Fan, K., Delgado-baquerizo, M., Guo, X., Wang, D., Wu, Y., Zhu, M., Yu, W., 2019. Suppressed N fixation and diazotrophs after four decades of fertilization. Microbiome 7, 1–10.
Freedman, Z., Eisenlord, S.D., Zak, D.R., Xue, K., He, Z., Zhou, J., 2013. Towards a molecular understanding of N cycling in northern hardwood forests under future rates of N deposition. Soil Biology and Biochemistry 66, 130–138.
Girkin, N.T., Turner, B.L., Ostle, N., Craigon, J., Sjögersten, S., 2018a. Root exudate analogues accelerate CO2 and CH4 production in tropical peat. Soil Biology and Biochemistry 117, 48–55.
Girkin, N.T., Turner, B.L., Ostle, N., Sjögersten, S., 2018b. Composition and concentration of root exudate analogues regulate greenhouse gas fluxes from tropical peat. Soil Biology and Biochemistry 127, 280–285.
Gramss, G., Voigt, K.D., Bublitz, F., Bergmann, H., 2003. Increased solubility of (heavy) metals in soil during microbial transformations of sucrose and casein amendments. Journal of Basic Microbiology 43, 483–498.
Hart, S.C., Stark, J.M., Davidson, E.A., Fristione, M.K., 1994. Nitrogen mineralization immobilization and nitrification. In: Methods of Soil Analysis Part 2 Microbiiological and Biochemical Properties, pp. 985–1018.
Hinsinger, P., Plassard, C., Tang, C., Jaillard, B., 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant and Soil 248, 43–59.
Jenkinson, D.S., Brookes, P.C., Powlson, D.S., 2004. Measuring soil microbial biomass. Soil Biology and Biochemistry 36, 5–7.
Jilling, A., Kane, D., Williams, A., Yannarell, A.C., Davis, A., Jordan, N.R., Koide, R.T., Mortensen, D.A., Smith, R.G., Snapp, S.S., Spokas, K.A., Stuart Grandy, A., 2020. Rapid and distinct responses of particulate and mineral-associated organic nitrogen to conservation tillage and cover crops. Geoderma 359, 114001.
Jilling, A., Keiluweit, M., Contosta, A.R., Frey, S., Schimel, J., Schnecker, J., Smith, R.G., Tiemann, L., Grandy, A.S., 2018. Minerals in the rhizosphere: overlooked mediators of soil nitrogen availability to plants and microbes. Biogeochemistry 139, 103–122.
Kang, S., Post, W., Wang, D., Nichols, J., Bandaru, V., West, T., 2013. Hierarchical marginal land assessment for land use planning. Land Use Policy 30, 106–113.
Kasmerchak, C.S., Schaetzl, R., 2018. Soils of the GLBRC Marginal Land Experiment (MLE) Sites 1–14.
Keiluweit, M., Bougoure, J.J., Nico, P.S., Pett-Ridge, J., Weber, P.K., Kleber, M., 2015. Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change 5, 588–595.
Landi, L., Valori, F., Ascher, J., Renella, G., Falchini, L., Nannipieri, P., 2006. Root exudate effects on the bacterial communities, CO2 evolution, nitrogen transformations and ATP content of rhizosphere and bulk soils. Soil Biology and Biochemistry 38, 509–516.
Le Roux, M.R., Khan, S., Valentine, A.J., 2008. Organic acid accumulation may inhibit N2 fixation in phosphorus-stressed lupin nodules. New Phytologist 177, 956–964.
Li, B., Li, Y.Y., Wu, H.M., Zhang, F.F., Li, C.J., Li, X.X., Lambers, H., Li, L., 2016. Root exudates drive interspecific facilitation by enhancing nodulation and N2 fixation. Proceedings of the National Academy of Sciences of the United States of America 113, 6496–6501.
Liu, Y., He, N., Zhu, J., Xu, L., Yu, G., Niu, S., Sun, X., Wen, X., 2017. Regional variation in the temperature sensitivity of soil organic matter decomposition in China's forests and grasslands. Global Change Biology 23, 3393–3402.
Meier, I.C., Finzi, A.C., Phillips, R.P., 2017. Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. Soil Biology and Biochemistry 106, 119–128.
Plante, A.F., Conant, R.T., Stewart, C.E., Paustian, K., Six, J., 2006. Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. Soil Science Society of America Journal 70, 287–296.
Rao, V.R., 1978. Effect of carbon sources on asymbiotic nitrogen fixation in a paddy soil. Soil Biology and Biochemistry 10, 319–321.
Reed, S.C., Townsend, A.R., Cleveland, C.C., Nemergut, D.R., 2010. Microbial community shifts influence patterns in tropical forest nitrogen fixation. Oecologia 164, 521–531.
Rodrigues, R.R., Moon, J., Zhao, B., Williams, M.A., 2017. Microbial communities and diazotrophic activity differ in the root-zone of Alamo and Dacotah switchgrass feedstocks. GCB Bioenergy 9, 1057–1070.
Roley, S.S., Duncan, D.S., Liang, D., Garoutte, A., Jackson, R.D., Tiedje, J.M., Robertson, G.P., 2018. Associative nitrogen fixation (ANF) in switchgrass (Panicum virgatum) across a nitrogen input gradient. PLoS One 13, e0197320.
Roth, V.N., Dittmar, T., Gaupp, R., Gleixner, G., 2015. The molecular composition of dissolved organic matter in forest soils as a function of pH and temperature. PLoS One 10, 1–23.
Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology 75, 1589–1596.
Ruan, L., Bhardwaj, A.K., Hamilton, S.K., Robertson, G.P., 2016. Nitrogen fertilization challenges the climate benefit of cellulosic biofuels. Environmental Research Letters 11, 064007.
Shi, S., Richardson, A.E., O'Callaghan, M., Deangelis, K.M., Jones, E.E., Stewart, A., Firestone, M.K., Condron, L.M., 2011. Effects of selected root exudate components on soil bacterial communities. FEMS Microbiology Ecology 77, 600–610.

Sinsabaugh, R.L., Reynolds, H., Long, T.M., 2000. Rapid assay for amidohydrolase (urease) activity in environmental samples. Soil Biology and Biochemistry 32, 2095–2097.
Smercina, D.N., Bowsher, A.W., Evans, S.E., Friesen, M.L., Eder, E.K., Hoyt, D.W., Tiemann, L.K., 2020a. Switchgrass rhizosphere metabolite chemistry driven by nitrogen availability. Phytobiomes J. 5, 88–96.
Smercina, D.N., Evans, S.E., Friesen, M.L., Tiemann, L.K., 2020b. Impacts of nitrogen addition on switchgrass root-associated diazotrophic community structure and function. FEMS Microbiology Ecology 96, fiaa208.
Smercina, D.N., Evans, S.E., Friesen, M.L., Tiemann, L.K., 2019a. To fix or not to fix : controls on free-living nitrogen fixation in the rhizosphere. Applied and Environmental Microbiology 85 e02546-18.
Smercina, D.N., Evans, S.E., Friesen, M.L., Tiemann, L.K., 2019b. Optimization of the 15N2 incorporation and acetylene reduction methods for free-living nitrogen fixation. Plant and Soil 445, 595–611.
Smercina, D.N., Evans, S.E., Friesen, M.L., Tiemann, L.K., 2021. Temporal dynamics of free-living nitrogen fixation in the switchgrass rhizosphere. Global Change Biol. Bioenergy 1–17, 00.
Sokol, N.W., Bradford, M.A., 2019. Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. Nature Geoscience 12, 46–53.
Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry 19, 703–707.
Wepking, C., Badgley, B., Barrett, J., Knowlton, K., Lucas, J., Minick, K., Ray, P., Shawver, S., Strickland, M., 2019. Prolonged exposure to manure from livestock administered antibiotics decreases ecosystem carbon-use efficiency and alters nitrogen cycling. Ecology Letters 22, 2067–2076.
Wickham, H., 2016. ggplot2 - Elegant Graphics for Data Analysis, second ed. Springer-Verlag, New York.
Yu, G., Xiao, J., Hu, S., Polizzotto, M.L., Zhao, F., McGrath, S.P., Li, H., Ran, W., Shen, Q., 2017. Mineral availability as a key regulator of soil carbon storage. Environmental Science and Technology 51, 4960–4969.
Yu, H., Huang, G.H., 2009. Effects of sodium acetate as a pH control amendment on the composting of food waste. Bioresource Technology 100, 2005–2011.