Plant intraspecific competition and growth stage alter carbon and nitrogen mineralization in the rhizosphere

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Abstract

Plant roots interact with rhizosphere microbes to accelerate soil organic matter (SOM) mineralization and promote nutrient acquisition. Root-mediated changes in SOM turnover largely depend on root-C input and soil nutrient availability. Hence, interspecific competition and nutrient uptake dynamics over plant development stages as well as spatiotemporal variability in C input may modify SOM turnover. To investigate the effect of intraspecific competition on SOM mineralization at three growth stages (heading, flowering and ripening), we grew maize (C4 plant) under three planting densities on a C3 soil. 13C-natural abundance and 15N-pool dilution were applied in situ to determine C- and N-mineralization rates. Soil C- and N-mineralization rates were tightly coupled and peaked at maize flowering. However, the C-to-N-mineralization ratio increased with N, indicating that microbes mineralize N-rich components to mine SOM for N. Furthermore, intraspecific competition did not affect root biomass; instead, plants shaped root morphology towards higher specific root length as an efficient strategy competing for nutrient. Hence, root morphologic traits rather than root biomass per se were positively related to C- and N-mineralization. Overall, plant competition for nutrients controlled the intensity and mechanisms of soil C- and N-turnover by the adaptation of root traits and nutrient depletion.

Keywords: Soil organic matter decomposition, carbon and nitrogen cycling, microbial activation, maize, plant intraspecific competition, root trait, arable soil

1. Introduction

The coexistence of organisms in unfertilized soils is characterized by strong competition for nutrients between plant communities and between plant roots and microorganisms (Kuzyakov & Xu 2013; Moreau *et al.* 2015; Adler *et al.* 2018). Soil organic matter (SOM) is a major reservoir of essential nutrients required for plant growth, but the availability of these nutrients, notably nitrogen (N), largely relies on microbial mediated transformation. Hence, microbial N mineralization from SOM, and subsequent immobilization/nitrification are crucial processes determining the intrinsic N supply of soil for plant productivity in natural and agroecosystems (Tiessen, Cuevas & Chacon 1994). The microbially driven processes can be significantly regulated by plant roots, as root-derived inputs of labile organic compounds (i.e., rhizodeposits) fuel the activity and growth of heterotrophic microorganisms (Paterson 2003; Cheng & Kuzyakov 2005). This, in turn, stimulates the synthesis of extracellular enzymes to mine for nutrients necessary for microbial growth, causing the acceleration of SOM mineralization (microbial N mining hypothesis; Craine et al., 2007). As a result, microbial N mineralization and immobilization tend to increase (Zhu *et al.* 2014; Murphy, Baggs, Morley, Wall &

Paterson 2015; Ehtesham & Bengtson 2017), facilitating plant N uptake (Dijkstra, Bader, Johnson & Cheng 2009; Frank & Groffman 2009). Such root-mediated changes in SOM mineralization have increasingly been recognized as a nutrient acquisition strategy of plants to exchange carbon (C) to soil microorganisms for N and other nutrients (Kuzyakov & Xu 2013). Hence, to consider the processes by which plant roots govern SOM turnover has far-reaching implications for understanding plant-microbial-soil interactions in terrestrial ecosystems.

Previous studies have shown that root-mediated changes in SOM mineralization vary with plant growth stages and plant-plant interactions (Cheng *et al.* 2014; Huo, Luo & Cheng 2017). Root physiological and morphological traits are major factors regulating SOM turnover, such as root biomass (Dijkstra & Cheng 2007), the quality and quantity of root exudates (Zhu & Cheng 2012), root architecture and morphology (Pausch*et al.* 2016). Root traits are spatially and temporally dynamic, changing in response to its controlling factors such as light, soil nutrients and water content (Kuzyakov *et al.* 2002; Craine, Wedin, Chapin & Reich 2003; Sanaullah, Chabbi, Rumpel & Kuzyakov 2012), and associated plant performance (i.e., photosynthesis) throughout the growth stages (Bardgett, Bowman, Kaufmann & Schmidt 2005). The alterations of root traits may shape microbial function and consequently modify SOM mineralization, through the changes in the abundance of available C and nutrients and in soil physical and chemical properties, i.e., water, pH values and soil aggregation (Shields, Paul, Lowe & Parkinson 1973; Jenkinson & Rayner 1977).

Plant-plant interactions often impose interspecific and intraspecific competition for above and belowground resources, i.e., light and nutrients. However, the effect of plant-plant interactions on soil C and N turnover through rhizosphere processes remains uncertain, since contradictory results have been shown previously (Fan, Zhang & Lu 2011; Pausch, Zhu, Kuzyakov & Cheng 2013; Yin, Dijkstra, Wang, Zhu & Cheng 2018). With increased plant competition for nutrients, the plant may increase C allocation to roots and adjust root physiological and morphological traits that enhance their competitive capacity relative to their neighbors, thereby potentially enhancing the C-N exchanges and SOM mineralization (Tilman 1990; Schenk 2006; Kunstler *et al.* 2016). In contrast, it has previously been reported that SOM mineralization can also be suppressed under the interspecific (Dijkstra, Morgan, Blumenthal & Follett 2010; Pausch *et al.* 2013) and intraspecific competition (Yin et al., 2018). These negative effects are explained by the nutrient competition hypothesis (Dormaar, 1990; Kuzyakov, 2002), which suggests that strong competition for nutrients between roots and microorganisms may inhibit microbial activity for decomposing SOM due to nutrients limitation. In addition to nutrients, plants compete for light with denser canopies, leading to the uncertainty outcome of photo-assimilate supply to belowground and hence SOM mineralization (Aerts 1999; Wang *et al.* 2020). Despite several studies that have examined the effects of plant competition on SOM turnover, they were mostly conducted under controlled conditions with a restricted soil volume for nutrient uptake by plants (Dijkstra *et al.* 2010; Pausch *et al.* 2013; Yin *et al.* 2018; Schofield *et al.* 2019; Wang *et al.* 2020). Direct estimation of plant competition on SOM mineralization and the relevance of microbial mechanisms under field conditions still remain elusive.

In natural ecosystems, the microbial activation by roots and subsequent mining for N from SOM has been identified to be an essential driver for the coupling of C and N turnover of soils (Phillips, Finzi & Bernhardt 2011; Finzi *et al.* 2015). However, conventional agriculture often assumes that the N supply of N from SOM decomposition is inadequate to meet the plant demands of plants, especially for crops with a high N uptake rate, e.g. maize (Zea mays) (Loecke, Cambardella & Liebman 2012; Osterholz, Rinot, Liebman & Castellano 2017). Thus, maize has received much more N-fertilizers than other crops worldwide (FAO 2006). Intensive fertilization may hamper the reliance of plant N-uptake on N mineralization, causing the potential decoupling of soil C and N cycling (Drinkwater & Snapp 2007). Very few experiments have been conducted to examine the processes by which roots regulate SOM decomposition and N mineralization in the agriculture field, though it is essential in the context of fertilization management in the agroecosystem (Francis*et al.* 2003; Spiertz 2010).

Therefore, this study aims to investigate how plants control the coupling between C-input and SOM turnover for nutrient uptake in an arable soil under field condition, with a focus on assessing the temporal dynamics

at different plant growth stages and the effects of plant intraspecific competition. Experimental trials with varying plant densities were established. We grew maize (C4 plants) with three planting densities for 132 days on a C3 soil (C3-to-C4 vegetation change; Kumar, Kuzyakov & Pausch 2016). The ^{13}C natural abundance approach was used to partition total CO_2 efflux for SOM-derived CO_2 and root-derived CO_2, and *in situ* ^{15}N pool dilution was applied to quantify gross N transformation. Concurrently, soil and microbial properties, and root morphology were measured at three plant growth stages (heading, flowering and maize ripening). We hypothesize that i) root mediated changes in SOM decomposition are associated with N mineralization during plant growth because of microbial activation by roots for N mining, and ii) the modulation of SOM decomposition and N mineralization by intraspecific competition depends on the root traits for nutrient uptake and the soil mineral nutrient status.

2. Materials and methods

2.1 Study site

The experiment was conducted on an agricultural field at the "Reinshof" research station of the Georg-August University of Göttingen (51°29'37.2"N and 9deg55'36.9"E). The study area has a temperate oceanic climate with an annual mean temperature of 8.5 degC and a mean precipitation of 850 mm. The meteorological parameters during the experimental monitoring period are shown in Fig. S1. The soil is classified as a Haplic Luisol with a silty loam texture (74.9% silt, 3.2% sand, 21.9% clay) (Berger 1999). It contains 1.41 +- 0.04% total C and 0.16 +- 0.002% total N and has a bulk density of about 1.3 g cm^{-3} from 0-35 cm depth and a pH of 6.2 (Kumar, Dorodnikov, Splettstosser, Kuzyakov & Pausch 2017; Mason-Jones, Schmucker & Kuzyakov 2018). The organic C at the site originates from permanent C3 vegetation ($\delta^{13}C$ of soil = -25.1 separate root-derived C from SOM-derived C, a vegetation change from C_3 to C_4 (maize; *Zea mays L. cv. Colisee* ; $\delta^{13}C$ of maize = -13.3 This allowed introducing a distinct ^{13}C signal into the soil with a difference in $\delta^{13}C$ values between soil and plant is > 11.8

2.2 Experimental setup

Sixteen experimental plots (each with an area of 5 × 5 m) were aligned in 4 rows in the field, each with 2-m wide buffer stripes to exclude the neighboring effects. A gradient of plant densities was established following a random design, with 4 replicates each: 1) a plant density of 5 plants m^{-2} as the control (P); 2) a double plant density of 10 plants m^{-2} (DP), which is equivalent to the common maize plant density for conventional farming in Germany; 3) a triple plant density of 15 plants m^{-2} (TP). Additionally, four plots were kept free from vegetation as bare fallow.

Maize seeds were firstly sown in plant-treated plots with a density of 15 plants m^{-2}. They were manually thinned to the respective low and double plant density 30 days after planting (DAP). Before maize sowing, conventional tillage practices were operated up to 30 cm of soil depth and all plots received phosphorus (P) and potassium (K) fertilizers. Temperature sensors (32 PT-100 sensors) were installed at 10 cm depth to monitor soil temperature. Soil moisture (0-10 cm depth) was measured by 6 ECH2O EC-5 moisture sensors (decagon devices). Hourly air temperature and atmospheric pressure were obtained from the weather station of the German Weather Service in Göttingen.

2.3 Sampling and analyses

2.3.1 Plant and soil sampling

Plants and soil were sampled at 72, 102 and 30 days after planting. The shoots of the plants were randomly sampled from each plot and weighed after oven-drying at 60 °C for 48 hours. Since the roots of maize plants are mainly concentrated in the upper 30 cm (Amos & Walters 2006), soils and roots were collected together by soil cores (˜7 cm diameter) from four soil depths at 0–5 cm, 5–15 cm, 15–25 cm and 25–35 cm in the middle of the diagonal between two plants. In the lab, root samples were separated from soils by sieving and were washed. Roots were then scanned on a flatbed scanner and analyzed for length and diameter (WinRhizo, Regent Instruments Inc., Quebec City, Canada). After scanning, the roots were dried at 60 °C for 48 hours and weighed.

Microbial biomass C (MBC) and microbial biomass N (MBN) were determined for all depth at each of the three sampling times by the chloroform fumigation-extraction method with modifications (Vance, Brookes & Jenkinson 1987). Briefly, 8 g of fresh soil was extracted with 40 mL of 0.05 M K_2SO_4 after shaking for 60 min on a reciprocating shaker (Laboratory shaker, GFL 3016) and the filtrates were measured for total extractable C and N with a multi C/N analyzer (multi C/N analyzer 2100S, Analytik, Jena). The same extraction procedure was used for fumigated soil, which was fumigated with ethanol-free $CHCl_3$ at room temperature for 24 h. Extractable organic C and N of non-fumigated soil were used as a measure of dissolved organic C (DOC) and dissolved N (DN). MBC and MBN were calculated as a difference of total extractable organic C and N between fumigated and non-fumigated samples using the extraction efficiency of 0.45 and 0.54 for C and N, respectively (Joergensen & Mueller 1996). Besides, 10 g soil of each core was oven-dried at 105°C for 24h to determine the gravimetric water content.

2.3.2 Respiration measurements

From July to late October 2015, soil CO_2 efflux was measured *in situ* using pre-installed soil chambers at 54, 74, 90, 102, 122, 132 days after the planting. The vented static chambers made of polyvinyl chloride (area 0.05 m^2 and approx. 14.5 L total volume) were inserted ˜2 cm into the soil in the center of each plot for the entire measurement period. Concurrently, 20 mL gas samples were collected at 20-min intervals using a syringe and stored in pre-evacuated exetainer vials with rubber septa (Exetainer; Labco Limited, Lampeter, UK) and analyzed for the $\delta^{13}C$ of CO_2 using an isotope ratio mass spectrometer (IRMS) (Finnigan Delta plus XP, Thermo Electron Corporation, Germany).

2.3.3 Gross N mineralization and nitrification

Gross rates of N mineralization and nitrification were measured *in situ* three times at 74, 102, and 132 days' after planting, which corresponds to the heading, flowering and ripening stages of maize plants (Meier 2001). The ^{15}N pool dilution approach by intact soil cores was used to estimate gross N mineralization (GNM) and gross nitrification rates (GNN) (Davidson, Hart, Shanks & Firestone 1991; Hart, Nason, David D & A 1994). In each plot, five intact soil cores (5 cm high with a volume of 251.2 cm^3) were taken: $^{15}NH_4Cl$ solution consisting of a mixture of 0.6 μg ^{15}N g^{-1} soil (99 atom% ^{13}C, Sigma Aldrich) and 2.4 μg^{14}N g^{-1} soil, was added to two of the five cores for determining gross N mineralization; $K^{15}NO_3$ solution, a mixture of 0.6 μg^{15}N g^{-1} soil (99 atom%^{13}C, Sigma Aldrich) and 2.4 μg ^{14}N g^{-1} soil, were added to another two cores with for determining gross nitrification. Water was added to the remaining cores for measuring the initial level of NH_4^+ and NO_3^-. The ^{15}N enrichments for $^{15}NH_4Cl$ (or $K^{15}NO_3$) solutions were 20 atom%. One of each set of cores was well mixed and extracted 10 minutes after^{15}N labeling (T0 soil cores), while the others from each pair were extracted after 24 of incubation (T1 soil cores). Briefly, 80 g soil from each core was extracted with 210 ml of 0.05 mol L^{-1} K_2SO_4 after shaking for 60 min on a reciprocating shaker (Laboratory shaker, GFL 3016). The concentrations of NO_3^- and exchangeable NH_4^+ in extracts were measured with a continuous flow analyzer (Skalar Analytical, Breda, Netherlands). Then, the ^{15}N enrichment of NH_4^+ and NO_3^- were determined by IRMS (Finnigan Delta plus XP, Thermo Electron Corporation, Germany) following the diffusion procedures (Murphy *et al.* 2003).

2.4 Calculations and statistics

The soil CO_2 efflux rates are calculated as the slope of linear regressions describing the change in CO_2concentration in the chamber headspace over time and are adjusted to field-measured air temperature and pressure during measurement. We used Keeling-Plots (Miller & Tans 2003) to calculate the $\delta^{13}C$ values of pure soil CO_2 without the admixture of atmospheric CO_2. Afterward, a linear two-source isotopic mixing model (Phillips & Gregg 2001) was applied to partition total CO_2 efflux into its sources, SOM- and root-derived CO_2. Gross N mineralization and gross nitrification were calculated following the equations in (Davidson*et al.* 1991; Sun, Schleuss, Pausch, Xu & Kuzyakov 2018).

To assess the potential intensity of the shoot and root competition, we use the modified version of relative competition intensity (RCI) according to (Callaway *et al.* 2002):

RCI = (X c - X t) / x,

where X c and X t is the shoot biomass (or root biomass, g m^{-2}) in the control (P) and density treatments (DP and TP), respectively, and x is the highest value of (X c:X t). Positive RCI value denotes competition.

Statistical analysis

The experiment was conducted with 4 field replicates. Normality (Shapiro-Wilk test, $p > 0.05$) and homogeneity of variance (Levene test, $p > 0.05$) were examined and data were log-transformed prior to analysis if necessary. We implemented two-way ANOVA with plant growth stages as the first factor and plant density as the second factor to identify single and their interacting effects on the total CO_2 efflux, root-derived CO_2 efflux, SOM-derived CO_2 efflux, GNM, specific root length and root-derived CO_2 efflux. However, two-way ANOVA revealed hardly any interactions between plant growth stages and plant density, and plant growth stages to be the predominant factor controlling the rate of C and N cycling. Therefore, we test additionly the independent effects of plant growth stages and plant density with a *post hoc* unequal N Tukey-Kramer significant difference (HSD). For plant and soil parameters (Table 1 and 2), we first used two way ANOVA to assess the effects of planting density, growth stages and their interactions. In addition, significant differences of those parameters among the density treatment at each growth stage were obtained by a one-way ANOVA analysis. We used a one-tailed t -test to assess the significances between RCI and zero. All statistical analyses were performed with SPSS 22, with the significance level at $p < 0.05$. Simple regressions were used to identify relationships between response variables with significances at $p < 0.05$.

3. Results

3.1 Plant biomass and root morphology

The shoot biomass per m^{-2} increased from maize heading to ripening stages. Root biomass per m^{-2} remained unchanged with growth stages for the single and double densities, but it increased for the triple density (Table 1). For each growth stage, maize produced a similar shoot and root biomass in total at the double and triple planting densities, which were higher than that at the single density (Table 1). However, both shoot and root biomass decreased after normalizing for planting density at both double and triple planting densities, and this was consistent with the positive relative competition intensity (RCI) of shoots and roots (Table 1), indicating the strong above- and belowground intraspecific competition with increased planting density. For either double or triple planting density, a higher belowground intraspecific competition (as indicated by RCI of roots) was reached at flowering. The RCI of root at the triple planting density was higher than the double planting density (Table 1), and was negatively correlated to mineral N concentrations in soil (R^2 = 0.6, $p = 0.03$; Fig. S2a).

For each planting density, maize had a higher root length density (RLD; in the upper 35 cm soil) at flowering stages. The RLD at the double and triple planting densities were averagely 1.7 and 2.2 times higher than the single planting density, respectively (across all growth stages; Table 1). At the heading and ripening stages, root length per unit of root biomass (specific root length; SRL) was similar among the planting densities, but the SRL at maize flowering stage increased by 46% and 49% at the double and triple densities, respectively (as compared with single density; Fig. 2a). Moreover, SRL increased with the RCI of root for maize at both double ($R^2 = 0.7$, $p = 0.001$) and triple densities ($R^2 = 0.3$, $p = 0.05$) (Fig. S2b).

3.2 Fluxes and sources of CO_2

Total CO_2 efflux ranged from 75 to 251 mg C m^{-2} day^{-1}. It remained similar at the heading and flowering stages and then decreased by 64% at maize ripening, irrespective of planting density (Fig. 1a). The contributions of root- and SOM-derived sources to total CO_2 efflux was similar between the planting densities but was dependent on maize growth stages. The root-derived CO_2 averagely contributed 56%, 28% and 46% to the total CO_2 at heading, flowering, and ripening stages, respectively (across planting density; Fig. 1a). Root-derived CO_2 efflux significantly decreased from the heading to ripening (ranged from 39.8 to 135 mg C $m^{-2}day^{-1}$) independently of the planting density (Fig. 1b). Root-derived CO_2 per unit of root biomass (specific root-derived CO_2) declined with maize growth stages, and the double and triple planting densities led

to lower specific root-derived CO_2 effluxes than single density at the heading and flowering stages (Fig. 2b). SOM-derived CO_2efflux was higher at maize flowering stage, followed by the heading and ripening stages for all levels of planting densities (ranged from 35.5 to 195.1 mg C m^{-2} day^{-1}; Fig. 2c), but the planting density had a minor effect on SOM-derived CO_2 efflux for each growth stage (Fig. 2c).

3.3 Soil N mineralization and nitrification

Maize growth stages, but not the planting density exerted significant control over gross N mineralization (GNM), which varied between 41.9 and 88.2 mg N m^{-2} day^{-1} (Fig. 2d). A higher rate of GNM occurred at maize flowering and it remained similar at the heading and ripening stages across all planting densities. Likewise, there was no difference between the planting density in gross nitrification (GNN) and the higher GNN at the flowering stage compared to other stages (Table 2). The GNN rates at heading and flowering stages were higher than the rates of GNM. Mineral N concentrations (exchangeable NH_4^+ plus NO_3^-) averagely decreased with maize growth by 40% at the flowering and 30% at ripening as compared with heading stages (across overall planting densities; Table 2).

3.4 Soil C and N content and microbial biomass

Dissolved N (DN) concentration in soil decreased with maize growth. For each growth stage, the double and triple densities led to similar amounts of DN contents, which were approximately 1.7 times lower than that at the single density (Table 2). Dissolved organic C (DOC) concentration remained similar at the heading and ripening, but decreased at maize flowering (Table 2). Soil microbial biomass C (MBC) gradually increased from the heading to ripening stages and the increase was up to 57%, 12% and 62% at the single, double and triple densities, respectively, while microbial biomass N (MBN) was independent on maize growth (Table 2).

3.5 Relationships between soil C and N mineralization

There was a positive linear relation between SOM-derived CO_2 and GNM across all planting densities ($R^2 = 0.4$, $p < 0.04$; Fig. 3a), indicating that the SOM-C mineralization is highly correlated with N mineralization. The C: N ratio of SOM mineralization (the ratio between SOM-derived CO_2 and GNM) declined with growth stages, and it was reduced with increased planting densities compared to the single density at the earlier two growth stages (Table 2). Moreover, the C:N ratio of SOM mineralization increased with soil DN concentration ($r^2 = 0.3$, $p = 0.02$; Fig. 3b). SOM-derived CO_2 and GNM were independent on root biomass (Fig. S3a, b) and specific root-derived CO_2 efflux (Fig. S3c, d). However, both SOM-derived CO_2 and gross N mineralization increased with specific root length for the double and triple planting densities (Fig. 3a, b), indicating the root morphology modulated soil C and N turnover at higher planting densities.

4. Discussion

4.1 Rhizosphere effects on soil C and N mineralization depend on plant phenology

For all levels of planting densities, the SOM-C and N mineralization showed similar unimodal patterns throughout maize growth stages, with the higher rates at flowering (Fig. 1c). This indicates that plant development stages exert an important control on the dynamic of soil C and N, as similarly reported by earlier studies on maize (Li *et al.* 2017; Kumar, Shahbaz, Blagodatskaya, Kuzyakov & Pausch 2018) and other crops (Cheng, Johnson & Fu 2003; Pausch *et al.* 2013; Zhu*et al.* 2018). The phenology-dependent effect on C and N fluxes from SOM is plausibly explained by plant-microbial interactions that are driven by (i) root growth and altered quality and quantity of rhizodeposits and (ii) changes in plant morphological traits with growth stages, and (ii) plant-associated changes in soil properties and nutrient status.

While root biomass remained almost constant from the heading to ripening stages (across all planting densities; Table 1), root-derived CO_2 efflux strongly declined (Fig. 1b), even after normalization for root biomass (specific root-derived CO_2, Fig. 2b). This indicates the decreased root respiration and indirectly a reduction of the amount of rhizodeposits with maize growth. A major reason for this could be that annual crops allocate more C belowground during early phases of vegetation stages, whereas the newly assimilated C remain in aboveground tissues for producing biomass and cobs at later growth stages, despite the increased

shoots likely producing more photo-assimilated C (Gregory & Atwell 1991; Pausch & Kuzyakov 2018; Chen, Palta, Wu & Siddique 2019). This down-regulated C translocation from shoots to roots with maize growth was indicated by a negative relationship between root-derived CO_2 efflux and shoot biomass (Fig. S4a).

At the maize heading stages, the higher root-mediated C release (as indicated by root-derived CO_2) was coincident with the relatively abundant mineral N in soil (Fig. 1b and 2b; Table 2). The microbial growth and activity may be promoted with a better supply of C and nutrients sources (Hessen *et al.* 2004). This condition, however, favored microbial community to directly utilize easily degradable rhizodeposits over recalcitrant SOM for C and energy requirements (preferential substrate utilization, Blagodatskaya et al., 2011; Hagedorn et al., 2003). This is indicated by the much larger contributions of root-derived CO_2 to total CO_2 efflux ($>$ 50% of total CO_2) at the heading than at the other two stages, with consequent lower SOM mineralization (for all planting densities, Fig. 1a). Likewise, microorganisms might preferably assimilate the available N source and thus decreased N mineralization. Therefore, preferential substrates utilization by microorganisms results in the lower SOM decomposition and gross N mineralization at the earlier development stages, in contrast to that at the other growth stages.

The rates of SOM-C and N mineralization were highest at maize flowering, though root-derived C inputs decreased substantially (Fig. 1b and 2b) with a simultaneous decline of soil available N (DN and mineral N contents, Table 2). One mechanistic explanation for the enhanced SOM-C and N mineralization could be that the microbial community accelerates SOM mineralization to mine for N (Craine *et al.* 2007; Chen, Senbayram & Blagodatsky 2014; Sun *et al.* 2018). The accelerated gross N mineralization that released additional ammonium further resulted in higher gross nitrification at the flowering (Table 1). Moreover, SOM-C mineralization was accompanied by slight increases in MBC and a significant reduction in DOC contents (for all levels of planting density; Table 2). Hence, the SOM-derived CO_2cannot be mostly originated from microbial overflow respiration and accelerated microbial turnover. Besides, microbial communities likely have switched to the consumption of available dissolved C in the soil to maintain their functionality to mineralize SOM regardless of the reduced root-derived C supply (Blagodatskaya *et al.* 2014). Another explanation could be that the maize at the flowering stage has adapted root morphology, i.e., longer root length per unit root biomass and larger root surface areas on average, which potentially improved microbial mineralization for N mining (see chapter 4.2. Fig 2a, Table 1).

At the maize ripening stage, the relative lower SOM-C and N mineralization are likely due to the biotic and abiotic factors that constrain microbial activity. Root-mediated microbial activation was possibly inhibited because of the very low inputs of root-derived C after maize maturity (Fig. 1b). This is supported by our studies showing that the stimulating effect of roots on the activities of C-, N- and P-acquiring enzymes were lower at maize maturity compared to the earlier stages (the presence vs. the absence of maize; Kumar *et al.*2018). Due to the depletion of soil available N (Table 2), the intensified plant–microbial competition for N could suppress microbial activity and hence SOM mineralization (Kuzyakov & Xu 2013). Furthermore, abiotic environmental conditions such as cooler temperature, have affected microbial activity and turnover directly (Price & Sowers 2004), but may also have altered plant-microbial interactions through changes in plant activity (e.g., photosynthetic activity, transpiration and nutrient uptake) (Nord & Lynch 2009), with respective feedbacks for microbial processes.

Taken together, SOM-C mineralization positively related to gross N mineralization throughout maize growth (across all planting densities; Fig. 3a), which agrees with previous studies on grassland and forest soils (Dijkstra *et al.* 2009; Phillips *et al.* 2011; Bengtson, Barker & Grayston 2012). This indicates that soil C and N cycles are tightly coupled in this arable soil. Furthermore, the C-to-N mineralization ratios of SOM tended to increase with soil DN contents (Fig. 3b), which suggests that the intensity of N-fluxes associated with SOM mineralization was dependent on microbial N availability. When soil mineral N was depleted by plant N uptake (as suggested by a negative relationship between plant biomass and soil mineral N, Fig. S4b), microorganisms likely acted much more on specific N-rich components of the heterogeneous SOM to mine N contained within (Murphy et al., 2015). We, therefore, conclude that microbial N mining hypothesis underlies the coupled turnover of C and N in this arable soil across plant growth stages.

4.2 Rhizosphere effects on soil C and N mineralization depending on intraspecific competition

In light of microbial activation by living roots (Cheng & Kuzyakov 2005; Blagodatskaya *et al.* 2014; Kumar *et al.* 2016), one would expect that, with higher plant density, the increased root biomass and on the other hand the rapid exhaustion of the available nutrients by root uptake may stimulate microorganisms to mine for nutrients from SOM. However, planting density did not affect the root-derived CO_2 efflux, and the SOM-C and gross N mineralization (when comparing one growth stage; Fig. 1). Also, neither C nor N mineralization showed a clear relation with root biomass and root-derived CO_2 efflux (across plant growth stages; Fig. S3). This is inconsistent with earlier studies suggesting that the stimulation of microbial decomposition is largely dependent on the root biomass and their associated rhizodeposits (e.g., Bengtson et al., 2012; Dijkstra et al., 2006; Shahzad et al., 2015; Wang et al., 2015; Yin et al., 2018). We suggest that a possible reason is that plants at higher densities expressed other traits such as root morphology in regulating microbial decomposition as a result of the intensified intraspecific competition.

Competition occurs when plant growth and nutrition are constrained by neighbors as a result of the reduction above- or belowground resources, such as light, water, and nutrients (Aerts 1999; Colom & Baucom 2019). Given the positive relative competition intensity (RCI) of shoots and roots under both double and triple planting densities (Table 1), it is clear that higher planting densities induced the intensive above- and belowground intraspecific competition throughout the growth stages. The competition belowground is more intensive at maize flowering (Table 1), as similarly shown by other maize fields (Li *et al.* 2019). Root plasticity in morphological traits could be decisive for competitive success for nutrients and water uptake (Kuzyakov & Xu 2013; Ahkami, Allen White, Handakumbura & Jansson 2017; Wen, Li, Shen & Rengel 2017). With increased intraspecific competition at maize flowering, plant altered morphological traits towards thinner and longer root per biomass unit, rather than producing more roots and exudates (as indicated by decreased specific root-derived CO_2; Fig. 2a). Such root morphology adjustment is an efficient way to occupy a larger soil volume for exploring temporal and spatial available resources (Li *et al.* 2019). Accordingly, specific root length tended to increase with belowground intraspecific competition (RCI of roots) with increasing planting densities (Fig. S2b).

Furthermore, root morphologic traits were largely responsible for soil C and N turnover at higher planting densities, since both SOM-C and N mineralization increased with specific root length for the double and triple planting density, expect for single density (Fig. 4). Here, we suggest two possible effects of root morphology in regulating microbial decomposition of SOM. First, the longer root length and larger surface-area-to-volume ratios may largely extend the distribution of rhizodeposits for fueling microorganisms and simultaneously causes an evener nutrient depletion, facilitating microbial nutrient mining. Second, root morphology is a vital driver affecting soil properties such as aggregations (Dorodnikov *et al.* 2009). The increasing plant densities enhanced the proportion of smaller aggregates size classes ($<$ 2000-250 μm), which favored microbial and enzymatic activities due to the better supply of water and substrates (Kumar *et al.* 2017).

In addition to intraspecific competition, higher planting density intensified the plant-microbial competition for nutrients and could retard microbial activity for SOM decomposition due to very strong nutrient limitations (nutrient competition hypothesis; Dormaar 1990; Kuzyakov 2002). This could be one mechanistic explanation for the unaffected SOM-C and N mineralization rates at higher planting densities as compared to those at low planting density (Dijkstra *et al.*2010; Pausch *et al.* 2013; Yin *et al.* 2018). However, given the stable microbial biomass and enzyme activities at increasing planting densities, we cannot explicitly confirm the negative effects of nutrient competition on SOM mineralization (Table 2, Kumar *et al.*2017). This might be attributed to the high initial nutrients contents in arable soils compared to those in natural ecosystems. Therefore, *in situ* tracer labeling (i.e., ^{15}N) for reliably quantifying the plant-microbial competition for nutrients is needed (Kuzyakov & Xu 2013). Future work also needs to encompass other competitive and mutualistic interactions such as N fixation by rhizobia and mycorrhizal fungi. In summary, plants modify root morphology to obtain a greater capacity to forage nutrients in response to intraspecific competition. This, in turn, affected microbial mineralization for SOM.

In conclusion, our study provides *in situ* evidence for the predominant mechanisms of rhizosphere effects on

soil C and N mineralization in an arable soil under field condition. Root-mediated acceleration of microbial activity and nutrient mining from SOM is a major mechanism driving C and N cycling as indicated by the coupled SOM-C and N mineralization throughout plant growth. The mineralization rates of C relative to N are regulated by microbial N availability and demand. Furthermore, owing to higher intraspecific competition at flowering, maize adjusted its root morphology for competing nutrients by the occupation of new soil volume, and strongly impact on SOM mineralization. Instead of root mass *per se* , SOM-C and N mineralization under higher planting densities were more related to root morphology (i.e., specific root length) (Fig. 5. Consequently, due to an elevated demand for nutrients under plant-plant and/or plant-microbial competition, root adaptation traits and soil nutrients availability plays an important role in modulating the activity and processes of microbial C and N cycling.

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Table 1: Plant shoot and root biomass, relative competition intensity, root length density and root surface area depending on plant growth stages and the planting densities.

Growth stages	Plant density	Biomass kg^{-1} m^{-2}	Biomass kg^{-1} m^{-2}	Relative competition intensity	Relative compe
		Shoot	Root	Shoot	Root
Heading	P	0.36 (0.05)b	0.01 (0.002)b		
74 DAP	DP	0.57 (0.04)a	0.03 (0.002)a	0.12 (0.05)b	0.02 (0.02)b
	TP	0.7 (0.05)a	0.02 (0.001)a	0.32 (0.05)a	0.46 (0.02)a
Flowering	P	1.08 (0.18)b	0.02 (0.001)		
102 DAP	DP	1.13 (1.10)b	0.02 (0.002)	0.37 (0.01)	0.58 (0.01)
	TP	1.64 (0.18)a	0.03 (0.004)	0.47 (0.06)	0.53 (0.06)
Ripening	P	1.53 (0.14)b	0.02 (0.001)b		
132DAP	DP	2.27 (0.25)a	0.03 (0.003)ab	0.17 (0.07)b	0.28 (0.08)
	TP	1.86 (0.21)ab	0.03 (0.004)a	0.57 (0.04)a	0.40 (0.09)
ANOVA p-values					
Density		0.001	0.004	<0.001	0.002
Growth stages		<0.001	0.03	0.003	<0.001
Density × stages		0.02	0.07	0.02	0.001

Data are means with the standard errors (n=4) except for shoot biomass where n=8. Significant differences among three planting densities at each plant growth stages are denoted by different letters (post hoc Tukey–Kramer honest test, $p < 0.05$).

Table 2: Gross nitrification (GNN), total dissolved organic C (DOC) and dissolved N (DN), microbial biomass C (MBC) and N (MBN), mineral N (exchangeable NH_4^+ plus NO_3^-), C: N ratio of SOM mineralization (SOM-derived CO_2: GNM), the DOC: DN ratio and the MBC: MBN ratio for each level of the planting density at different plant growth stages.

Growth stages	Density	GNN	DOC	MBC	DN	MBN	mineral N	SOM-C
		mg N m^{-2} d-1	g C m^{-2}	g C m^{-2}	g N m^{-2}	g N m^{-2}	g N m^{-2}	
Heading	P	110.1 (54.1)	14.1 (0.5)	47.9 (2.9)c	6.4 (0.7)a	11.6 (1.0)	2.1 (0.1)a	2.6 (0.6)
74 DAP	DP	46.4 (11.8)	16.2 (1.5)	68.9 (0.7)a	3.6 (0.02)b	11.5 (0.3)	2.0 (0.5)c	2.1 (0.7)
	TP	133.3 (46.4)	15 (0.7)	55.2 (0.5)b	3.8 (0.1)b	12.3 (1.0)	1.9 (0.1)b	1.3 (0.4)
Flowering	P	274.1 (125.3)	13.7 (0.8)	75.1 (1.9)	5.7 (0.5)a	12.1 (0.7)b	0.8 (0.1)	2.3 (0.2)
102 DAP	DP	124.6 (106.1)	13.7 (0.7)	79.6 (1.7)	3.9 (0.3)b	14 (0.4)a	1.1 (0.3)	1.7 (0.1)
	TP	170.4 (46.0)	13.3 (0.3)	75 (1.6)	3.8 (0.3)b	13.7 (0.5)ab	1.1 (0.1)	1.6 (0.2)
Ripening	P	-36.7 (28.0)	17.4 (0.01)	75.2 (3.9)b	4.9 (0.6)a	12.1 (0.3)	1.2 (0.1)	0.6 (0.1)
132DAP	DP	-51.4 (27.6)	17.4 (0.8)	77.2 (3.6)ab	3.9 (0.2)b	12.2 (1.4)	1.1 (0.3)	1.1 (0.2)
	TP	-1.8 (24.4)	18.1 (0.8)	89.6 (4.0)a	2.9 (0.1)b	13.8 (0.7)	1.0 (0.1)	0.8 (0.1)
ANOVA p-values								
Density		0.3	0.5	0.0005	<0.001	0.1	0.001	0.09
Growth stages		0.001	<0.001	<0.001	0.009	0.08	0.3	0.0002
Density × stages		0.7	0.6	0.0002	0.8	0.6	0.02	0.05

Data are means with standard errors (n=4). Significant differences among three planting densities at each plant growth stages are denoted by different letters (post hoc Tukey–Kramer honest test, $p< 0.05$).

Figure legends

Fig. 1. Total CO_2 efflux (a), root-derived (b) and SOM-derived CO_2 efflux (c), and gross N mineralization (GNM) (bars show means ± SE; n = 4) for the single, double and triple planting density at the heading, flowering, and ripening stages. Significant differences ($p < 0.05$) between growth stages for each planting density are presented by different uppercase letters, while lowercase letters indicate significant differences between three planting densities at each growth stage.

Fig. 2. Specific root length (a) and specific root-CO_2efflux (b) for the single, double and triple planting densities at maize heading, flowering and ripening stages (bars show means ± SE; n = 4). Significant differences ($p < 0.05$) between growth stages for each planting density are presented by uppercase letters, while lowercase letters indicate significant differences between three planting densities at each growth stage.

Fig. 3: Linear relationship between SOM-derived CO_2efflux and gross N mineralization (GNM) (a) (n = 36). A linear relationship between the SOM-C to N rate of mineralization (the ratio between SOM-derived CO_2 and GNM) and total dissolved N (DN) (b) (n = 36). Data for (a) and (b) include three planting densities across all three growth stages.

Fig. 4: Linear relationship of SOM-derived CO_2 efflux (a) and gross N mineralization (GNM) (b) with specific root length (n=12). Each dots represents the individual replicate of each planting density (P, DP and TP) across three growth stages.

Fig. 5: The changes in root length density and belowground intraspecific intensity from maize heading to ripening stages, and the dynamics of soil C and N fluxes across all level of planting densities.

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