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Carbon dioxide fluxes in soil profiles as affected by maize phenology and nitrogen fertilization in the semiarid Loess Plateau

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ABSTRACT

To better understand the responses of subsoil CO_2 to maize (*Zea mays* L.) phenology and N fertilization, a field experiment was conducted from 2014 to 2015 in the Changwu Agri-Ecological Station, Shaanxi, China. The experiment included four treatments: unplanted and N-unfertilized soil (C0), unplanted soil amended with 225 kg N ha^{-1} (CN), maize planted and N-unfertilized soil (P0), and maize planted soil fertilized with 225 kg N ha^{-1} (PN). Soil CO_2 concentration at 0–50 cm soil depth, at a resolution of 10 cm, was measured, and the CO_2 effluxes were calculated using the gradient method. Soil CO_2 concentrations and fluxes in the planted treatments corresponded with maize growth; they rapidly increased from the jointing stage, peaked around the milk stage, and then slowly decreased with plant maturity. CO_2 concentrations and fluxes in the planted soil were significantly higher compared to those in the unplanted soil. N inputs significantly decreased ($P < 0.05$) the CO_2 concentrations of the planted soil at depths of 10, 20, and 30 cm in 2015 and increased total CO_2 fluxes of the 0–50 cm soil layers during the maize growing season by 6% ($P = 0.29$) in 2014 and by 18% ($P < 0.01$) in 2015, with the cumulative plant-derived CO_2 fluxes enhanced by 20% ($P = 0.05$) and 29% ($P = 0.07$), respectively. In unplanted soil, the CO_2 concentrations and fluxes of the 10 cm soil layer were slightly ($P > 0.05$) decreased with N inputs in both years. The contributions of the plant-derived CO_2 effluxes to the total CO_2 effluxes of the 0–50 cm soil layers were affected by maize growth, with two year mean values of 0.49 for the P0 treatment and 0.55 for the PN treatment, respectively. The results indicated that subsoil CO_2 fluxes were affected by maize phenology and that application of N fertilizer enhanced subsoil CO_2 effluxes mainly by increasing plant-derived CO_2 effluxes during the late growing season.

1. Introduction

Soil plays a major role in the global C budget because it contains 2.3 times more C than the atmosphere (Lal, 2004). Carbon exchange between soils and the atmosphere is closely linked to global climate change because the C cycle is sensitive to environmental change and variations in the C cycle affect our climate (Van Groenigen et al., 2014). As the second-largest terrestrial carbon flux between soils and the atmosphere, soil respiration has been studied for several decades. However, the spatial and temporal heterogeneity of soil respiration is a major challenge in predictively modeling soil respiration and its components (Hopkins et al., 2013).

Soil respiration is a combination of plant-derived respiration (from root and microorganisms in the immediate vicinity of the roots) and microbial respiration derived from soil organic matter (SOM) (Kuzyakov, 2006). The rhizodeposition of living plants could greatly change (increase or decrease) the SOM decomposition, which is known as a rhizosphere priming effect. Therefore, the SOM-derived respiration includes CO_2 derived from decomposition of native SOM (basal respiration) and the additional SOM-derived CO_2 induced by the priming effect (Kuzyakov, 2002). Only measuring total soil CO_2 flux is confounded in evaluating soil C

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sequestration because only SOM-derived CO_2 contributes to changes in atmospheric CO_2 concentrations (Kuzyakov, 2006). Accordingly, separating plant-derived respiration from soil respiration could help us better understand the mechanisms of soil respiration and evaluate the C cycle of the terrestrial ecosystem. Various methods have been used to separate the estimation of plant-derived respiration from that of SOM-derived respiration associated with soil surface CO_2 emission (Kuzyakov, 2006); however, research on components of subsoil CO_2 is seldom reported.

Soil respiration is strongly affected by abiotic and biotic factors. Soil temperature and soil water content are the two major abiotic factors controlling soil respiration (Risk et al., 2002). Plants contribute to soil respiration by root respiration and to microbial respiration by the delivery of substrates (Philippot et al., 2009; Fender et al., 2013) and alter environmental factors indirectly (Yan et al., 2010). The contribution of plant-derived respiration also depends strongly on plant phenology (Fu et al., 2002; Gul and Whalen, 2013). For example, Sey et al. (2010) found the plant-derived respiration of corn and soybean to be the greatest during early vegetative growth, when greater C is allocated belowground (Qian et al., 1997; Fu et al., 2002). N fertilization, as a significant management strategy, could enhance crop yields (Liu et al., 2014) in the agriculture ecosystem and affect soil respiration by altering plant root growth (Shao et al., 2014) and microbial activities (Yan et al., 2010). However, N inputs have been reported to have different effects on soil CO_2 efflux (Mo et al., 2008; Ding et al., 2010; Sainju et al., 2010; Zhai et al., 2011). Moreover, the response of plant-derived and microbial respiration to N addition may not be consistent (Yan et al., 2010; Ni et al., 2012). Plant-derived respiration could be stimulated by the increase in root biomass (Shao et al., 2014; Zhang et al., 2014) or decreased by the reduction in belowground C allocation (Giardina et al., 2004; Olsson et al., 2005). N fertilization could also raise (Ding et al., 2010) or reduce (Ni et al., 2012) SOM-derived soil respiration, depending on the soil labile organic carbon content. The contribution of plant-derived CO_2 flux to soil CO_2 flux also affects the response of total soil CO_2 flux to N application (Hanson et al., 2000). Therefore, more detailed studies regarding the effect of N on soil respiration and its components are needed.

Net soil CO_2 flux (or soil respiration) is the result of CO_2 production in the soil and its transport within the soil and transfer across the soil surface to the atmosphere (Jassal et al., 2004). Traditionally, research has focused on soil surface CO_2 emission. However, obtaining information about CO_2 production, consumption and transport within a soil profile can help us better study the processes underlying soil CO_2 effluxes and take measures to reduce CO_2 emissions (Jassal et al., 2005). Subsurface approaches to soil CO_2 monitoring are becoming increasingly important for process studies in terrestrial carbon research. The soil vertical gradient measurement method, which assumes molecular diffusion is the most important gas transport pathway in soil, has gained widespread application and could be used to calculate the CO_2 flux between soil layers and between soil layers and the atmosphere based on CO_2 concentration and the effective gas diffusivity without disturbing the soil environment (Maier and Schack-Kirchner, 2014).

The Loess Plateau of Northwest China is a typical semiarid region where maize represents one of the major cereals grown widely (Liu et al., 2009). Maize has a high demand for N, and the addition of N at an appropriate rate can significantly promote maize growth and provide high grain yields (Liu et al., 2013; Bu et al., 2014). Undoubtedly, high yields rely on the acquisition of soil water and nutrients by roots. As large and extensive systems, most maize roots are concentrated in the top 30 cm layer of soil (Dwyer et al., 1996; Peng et al., 2012; Gao et al., 2014). Root activities logically affect the plant-derived CO_2 flux in this zone during the maize growing season. Moreover, some studies have suggested that CO_2 is produced mainly in the shallow soil layers (Jassal et al., 2005; Kusa et al., 2010; Xiao et al., 2015). In addition, our previous study indicated that CO_2 concentrations are relatively dynamic in the top 50 cm soil layer compared with those observed in deeper layers and that N addition could slightly increase CO_2 effluxes (Nan et al., 2016). In this study, we obtained continuous measurements of subsoil CO_2 concentration in the 0–50 cm soil layer, and based on these gradients, CO_2 effluxes within soil layers were calculated. Our objective was to determine (1) how the subsoil CO_2 effluxes are affected by maize phenology and (2) the response of subsoil CO_2 effluxes and its components to N addition. Our hypothesis was that the CO_2 in the subsoil could be affected by plant phenology and that N addition could increase plant-derived CO_2 effluxes within soil layers through improving maize growth.

2. Materials and methods

2.1. Site description

A two-year (2014 and 2015) field experiment was conducted at the Changwu Agri-Ecological Station (35.28°N,107.88°E, 1200 m altitude), which is located on the Loess Plateau, China. The site is characterized by a semiarid continental climate. The average annual precipitation is 584 mm, 73% of which occurs during the maize growing season (MS), and the annual potential evaporation is 1560 mm. The annual mean air temperature is 9.1 °C. Generally, the dominant cropping system in this area is one harvest a year, and the major cereal crops are winter wheat (*Triticum aestivum* L.) and spring maize (*Zea mays* L.). Agricultural production in this region completely depends on natural rainfall. The soil type at the study site is a Cumuli-Ustic Isohumosol (Gong et al., 2007), according to Chinese soil taxonomy. The soil is a loam (Cumulic Haplustoll; USDA Soil Taxonomy System) developed from wind-deposited loess, which is relatively uniform and has high permeability. Prior to this experiment, the experimental field had been used for winter wheat or spring maize production for a long time. The field underwent maize and soybean intercropping in the previous year. Soil characteristics in the top 50 cm are listed in Table 1.

Table 1
Soil charactersat depths of 0–50 cm at the experimental site before planting in 2014.

Depth (cm)	pH	Sand (%)	Silt (%)	Clay (%)	Dry bulk density ($g\,cm^{-3}$)	Total organic carbon ($g\,kg^{-1}$)	Total nitrogen ($g\,N\,kg^{-1}$)	Mineral nitrogen ($mg\,N\,kg^{-1}$)	Available phosphorus ($mg\,P\,kg^{-1}$)	Available potassium ($mg\,K\,kg^{-1}$)
10	8.1	38.8	40.1	21.2	1.32	8.8	1.10	21.0	22.6	160.7
20	8.2	37.0	41.2	21.7	1.34	7.7	1.00	22.1	20.4	134.9
30	8.3	34.5	42.4	23.2	1.42	6.6	0.85	13.8	10.2	118.5
40	8.3	32.9	42.9	24.1	1.38	5.7	0.75	10.1	3.1	122.9
50	8.3	33.5	42.6	23.9	1.40	5.6	0.71	9.4	1.5	116.3

2.2. Field experiments and crop management

A randomized block design consisting of three replicates was used, with an area of 7 m × 8 m for each plot. The field experiment started in 2014 and involved four treatments: unplanted without N (C0); unplanted soil with 225 kg N ha^{-1} N (CN) as urea; maize planted without N (P0); and maize planted with 225 kg N ha^{-1} N (PN) as urea. Calcium superphosphate (40 kg P ha^{-1}) and potassium sulfate (80 kg K_2O ha^{-1}) were applied to the soil surface and then plowed immediately in all treatments, whereas urea (N 46%) was applied at the rate equivalent to 225 kg N ha^{-1} in two splits before plowing and during the maize V12 stage in a ratio of 1:2. Before sowing, all mixed fertilizers were manually broadcast over the soil surface then tilled into the soil. Topdressed N (July 5, 2014 and July 3, 2015) was applied using a hole-sowing machine. Spring maize (*var. Pioneer* 335) was sown (April 30 in 2014 and April 26 in 2015) into 5 cm deep holes using a hand-powered hole-drilling machine at a density of 65 000 plants ha^{-1} and harvested on September 18, 2014 and September 13, 2015. The distances between adjacent rows and hills were 50 cm and 30 cm, respectively. Weeds were periodically removed by hand during the maize growing season.

2.3. Sample collection and measurements

2.3.1. CO_2 concentrations in the soil

Each multiple sampling tube (inner diameter 4.0 cm) was made of poly-vinyl chloride (PVC) and consisted of five independent soil-air equilibration samplers (Fig. 1) (Wang et al., 2013; Zhou et al., 2016). Individual samplers were isolated by PVC plates. The tubes were installed to collected gas at soil depths of 10, 20, 30, 40 and 50 cm. Each sampler had a perforated lower section with 16 small holes and was covered by nylon mesh (0.038 mm mesh size). A gas sampler was connected to the soil surface by an organic glass tubule (inner diameter 0.4 cm) with a plastic three-way stopcock.

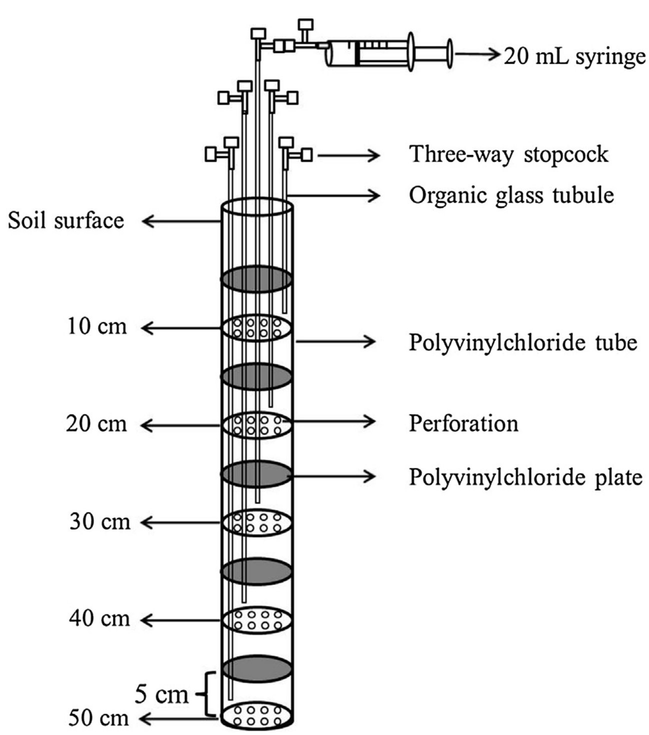

Fig. 1. Schematic diagram of the soil gas sampling system.

The three-way stopcocks were kept closed when not in use. After drilling holes using a hand auger with a diameter of 5.0 cm, the multiple sampling tubes were inserted into the holes with caution, and the space between the tubes and soil was back-filled layer by layer to minimize any disturbance. Soil gas sampling systems were installed at the center of each plot before maize sowing and were in place during the study. For the maize planted plots, gas sampling systems were placed between the maize rows.

Soil gas samples were measured weekly during the maize growing season and biweekly or monthly during the fallow season (FS). On each sampling day, the gas samples in the sampling tubes were withdrawn between 8:30 a.m. and 11:30 a.m. using 20 mL syringes equipped with three-way stopcocks. Prior to collecting gas, we pulled and pushed the syringes slowly three times to ensure the mixing of air inside the sampling systems. Soil surface air (0 cm) in each plot was collected concurrently. The gas samples were analyzed using a gas chromatograph (Agilent 7890A, Shanghai).

2.3.2. Chamber-based CO_2 fluxes measurements

CO_2 emissions were measured using the closed-chamber method. Each stainless steel chamber was composed of a 50 × 30 × 30 cm top chamber and a 50 × 30 × 15 cm base frame. The frames were inserted into the soil to a depth of 15 cm at the center of each plot before maize sowing and were in place during the sampling period unless they were removed for tillage events. The upper chamber had a 10 × 10 cm opening (for the maize plant) and consisted of two separate parts that were combined using hinges and airtight rubber seals. The bottoms of each part were also covered with airtight rubber seals. The upper chamber was coated with insulating material to minimize fluctuations of air temperature in the chamber during the sampling period. One fan positioned near the top of the chamber and pointed downward promoted the mixing of air inside the chamber. Each upper chamber was equipped with two ports: a silicon-sealed vent for sampling gas and another port for measuring the chamber temperature. Three maize seeds were sown in the center of the frame, and only one maize plant was left after thinning.

To collect gas samples, the top chambers were placed on the frames, and the chambers and frames were closely combined by two clamps. The opening allowed the maize to pass through the chamber top when the maize stalk was too high, and the space between the main stalk and the opening was filled with soft airtight materials when the chamber was closed. To ensure gas tightness, preservative films were wrapped around the stalk. Following checks for linearity of the CO_2 fluxes, gas samples were taken using 50 mL syringes 0, 10, 20 and 30 min after enclosure.

Surface flux samples we collected generally at the same time with the soil profile gas samples. The gas samples were analyzed using a gas chromatograph (Agilent 7890A, Shanghai, China) equipped with a flame ionization detector (FID). The FID was set at 250 °C to determine the CO_2 contents. Gas samples analyses were finished within 24 h on the sampling day.

2.3.3. Environmental and soil variables

The daily precipitation and daily mean air temperatures (1.5 m above the ground) were obtained from the Changwu Meteorological Monitoring Station, which is located within 50 m of the experimental site.

Temperature sensors were installed at soil depths of 10, 20, 30, 40 and 50 cm in each plot. For the planted plots, sensors were placed between the maize rows. The soil temperature was recorded using portable digital thermometers (JM624, Jinming Instrument Ltd., Tianjin, China) before and after collecting soil gas samples. The mean of the two readings was used as the soil temperature of the sampling day. Moreover, soil samples were taken in 10 cm increments from depths of 0 to 50 cm to determine water content weekly during the maize growing season and biweekly or monthly during the fallow season. However, soil water content was not measured when the soil was frozen (December to early March the following year). The soil samples were oven-dried at 105 °C to a constant weight to determine soil gravimetric water content, and the soil water-filled pore space (WFPS) was subsequently calculated.

Soil bulk density was measured by the cutting-ring method in the field. Soil particle size was analyzed by a Mastersizer 2000 laser particle-size analyzer (Malvern Inc., UK). Soil pH was measured using a soil/water ratio of 1:2.5. Soil organic C was measured by the dichromate oxidation method, and total N was analyzed by the Kjeldahl method. Fresh sub-samples were extracted with 1 M KCl, and the content of available nitrogen was analyzed using an automated flow injection analyzer (FlOWSYS, Italy). Soil available phosphorus was extracted in 0.5 mol L^{-1} $NaHCO_3$ and determined using the Olsen method. Soil available potassium content was determined by flame photometry.

2.3.4. Plant biomass sample

Maize developed at a similar rate with and without the application of N fertilizer; therefore, the sampling and measurement procedures were the same. Plant samples were collected at the 6-leaf-stage (V6), the 10-leaf-stage (V10), the silking stage (R1), the milk stage (R3), the dent stage (R5) and physiological maturity (R6). At each sampling, three adjacent plants in a row were cut as close as possible to the soil surface and then dried and weighed to measure the total aboveground biomass.

2.4. Calculations and statistical analyses

2.4.1. Soil water-filled pore space

The soil water-filled pore space (WFPS, %) was calculated as follows

$$\mathrm{WFPS} = \frac{\theta_m \times \rho_b}{1 - \frac{\rho_b}{\rho_s}} \times 100 \quad (1)$$

where θ_m is the soil gravimetric water content (%), ρ_b is the soil bulk density (g cm^{-3}), and ρ_s is the average soil particle density (2.65 g cm^{-3}).

2.4.2. CO_2 efflux in the soil profile

CO_2 effluxes were calculated using the following equation based on Fick's law (Marshall, 1959)

$$q = -D_\rho \frac{dc}{dz} \quad (2)$$

where q is the gas flux (g m^{-2} s^{-1}), positive values are defined as gas moving upward, and negative values as moving towards deeper layers. D_p is the effective diffusion coefficient of CO_2 in the soil (m^2 s^{-1}), and $\frac{dc}{dz}$ is the concentration gradient between two soil layers (g m^{-3} m^{-1}).

$$D_\rho = D_0 \varepsilon^{(1+C_m\Phi)} \left(\frac{\varepsilon}{\Phi}\right) \quad (3)$$

where D_0 is the CO_2 diffusivity in free air (m^2 s^{-1}), ε is the soil air-filled porosity (m^3 m^3), Φ is the soil porosity (m^3 m^3), and C_m is the media complexity factor. C_m is equal to 2.1 for intact soil (Moldrup et al., 2013). ε and Φ were calculated using the Millington-Quirk model (Millington and Quirk, 1961):

$$\Phi = 1 - \frac{\rho_b}{\rho_s}, \quad (4)$$

$$\varepsilon = \Phi - \theta_v \quad (5)$$

$$\theta_v = \theta_m \times \rho_b \quad (6)$$

where ρ_b is the soil bulk density (g cm^{-3}) and θ_v and θ_m are the soil volumetric water content (m^3/m^3) and gravimetric water content (g/g), respectively. D_0 is affected by temperature and pressure and can be estimated as follows:

$$D_0 = D_s \left(\frac{T}{T_0}\right)^{1.75} \left(\frac{P_0}{P}\right) \quad (7)$$

where T is the temperature (K), P is the air pressure (Pa), and D_s is a reference value at T_0 (273.15 K) and P_0 (1 atm), given as $1.39 \times 10^{-5} m^2 s^{-1}$ (Pritchard and Currie, 1982). For the CO_2 effluxes in the subsoil, we used the bottom depth below each layer (10, 20, 30, 40 and 50 cm) representing the whole soil layers (0–10, 10–20, 20–30, 30–40, and 40–50 cm) in the following tables and figures for convenience.

3.0.3. Cumulative gas effluxes

Cumulative emissions were calculated using the following formula:

$$T = \Sigma_{i=1}^{n} (X_i + X_{i+1})/2 \times (t_{i+1} - t_i) \times 24 \quad (8)$$

where T (kg ha^{-1}) is the cumulative CO_2 flux, X (kg ha^{-1} h^{-1}) is the average daily CO_2 flux, i is the ith measurement, and (t_{i+1}-t_i) is the number of days between two adjacent measurements.

Mean values of three replications are reported in the figures and tables. All statistics were carried out using SPSS 18.0. Statistically significant differences were identified using analysis of variance (ANOVA) and least significant difference (LSD) calculations at $P < 0.05$.

Herein, we refer to CO_2 derived from the microbial decomposition of soil organic matter (SOM) in unplanted soil as "SOM-derived respiration", and define the "plant-derived CO_2 flux" as the sum of root respiration, rhizo-microbial respiration and microbial respiration of living plant residues (Kuzyakov, 2006). Under the assumption that the plant-derived CO_2 flux was the difference between the maize planted soil and the unplanted soil (Hanson et al., 2000), the contribution of plant-derived effluxes to the total CO_2 effluxes (plant-derived effluxes ratio) was obtained by dividing the plant-derived cumulative CO_2 fluxes by the total cumulative CO_2 fluxes in the planted soil. It should be noticed that this method did not consider the priming effect and the discrepancy of abiotic environmental factors between the planted and unplanted soil.

Table 2
Average seasonal soil temperature (°C) at various soil depths under different treatments.

Soil depth (cm)	MS[a]–2014				FS				MS-2015			
	C0[b]	CN	P0	PN	C0	CN	P0	PN	C0	CN	P0	PN
10	20.0	19.9	18.7	18.9	4.9	4.8	4.4	4.4	19.3	19.3	18.7	18.4
20	20.2	20.1	18.9	19	5.2	5.2	4.7	4.9	19.5	19.1	18.8	18.6
30	20.4	20.5	19.3	19.3	5.6	5.7	5.3	5.4	19.7	19.8	19.0	19.0
40	20.5	20.5	19.4	19.4	6.2	6.1	6.0	5.9	19.6	19.6	18.8	19.0
50	20.2	20.1	19.1	19.1	6.5	6.6	6.3	6.2	19.7	19.6	18.8	18.8

[a] MS and FS denote the maize growing season and fallow season, respectively.
[b] C0, unplanted and N-unfertilized; CN, unplanted and N-fertilized; P0, maize planted and N-unfertilized; PN, maize planted and N-fertilized.

4. Results

4.1. Soil temperature, soil WFPS and CO_2 diffusivity in the soil

The soil temperature in the 10–50 cm soil layers is shown in Table 2. In contrast to that measured for the maize planted treatments, the soil temperature in the unplanted treatments was approximately 1 °C higher at various soil depths because the presence of maize decreased the direct solar radiation reaching the soil surface.

WFPS fluctuated with precipitation during the maize growing season (Fig. 2), especially in the top 10 cm. The annual precipitation was 597 mm in 2014, with 375 mm falling during the maize growing season, and the rainfall during the maize growing season in 2015 was 361 mm. Compared with that in the unplanted treatments, WFPS in the planted treatments was relatively lower at all soil depths after early June, when maize began to grow vigorously. The phenomenon was more distinct when less rainfall occurred from June 13 to July 26 in 2014. The average soil WFPS of the PN treatment was significantly lower than that of the P0 treatment at the depths of 10 and 20 cm in 2014 (Table 3). The mean WFPS of the PN treatment in 2015 was significantly ($P < 0.05$) lower than that of the P0 treatment at a depth of 0–50 cm during the maize growing season.

The effective diffusion coefficient of CO_2 in soil (D_p) varied over the season (Fig. 3), with larger values and fluctuations observed in the top 10 cm because the soil water content in the uppermost layer was highly dynamic. The D_p values of the planted treatments were higher than those of the unplanted treatments after early June due to decreased water content resulting from the consumption of water by rapidly growing plants, peaking between late July and early August in both years. Heavy rainfall events (e.g. 20 mm on 19 June 2014) dramatically reduced the D_p values. The D_p values of the P0 treatments were lower than those of the PN treatments, particularly in 2015, because N addition promoted maize growth and the consumption of more soil water, resulting in the relatively higher gas diffusion coefficients.

4.2. Soil CO_2 concentration and effluxes at different soil depths

4.2.1. Variation of CO_2 concentration in soil profile

Soil CO_2 concentrations varied seasonally and increased with soil depth throughout the measurement period (Fig. 4). The concentrations increased rapidly after planting, peaked in middle August and then declined towards winter. CO_2 pulses in the top 50 cm were observed after heavy rainfall, i.e., from 5 to 9 August in 2014, because water blocked soil pores and decreased gas diffusion rates (Fig. 3), therefore, CO_2 could not escape the soil. CO_2

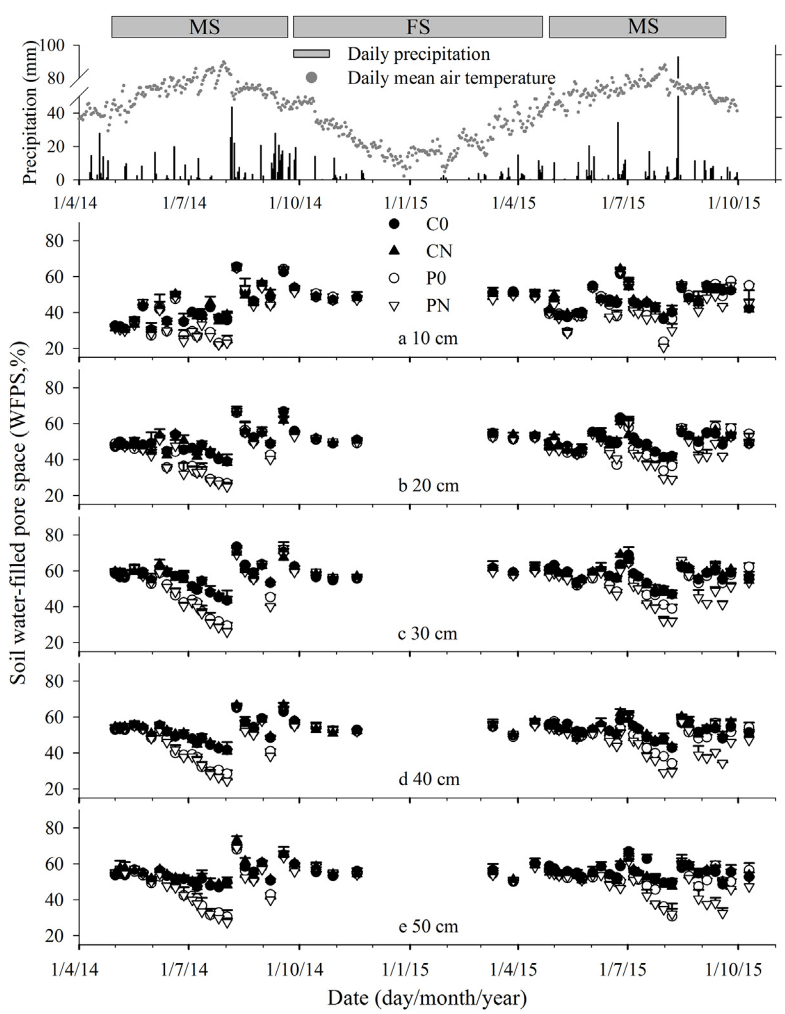

Fig. 2. Precipitation (mm) and soil water-filled pore space (WFPS, %) within soil profiles of different treatments during the study period. The bars represent the standard deviations of the means (n = 3). MS and FS denote the maize growing season and fallow season, respectively. C0, unplanted and N-unfertilized; CN, unplanted and N-fertilized; P0, maize planted and N-unfertilized; PN, maize planted and N-fertilized. Dotted lines indicate rainfall.

Table 3
Average seasonal soil WFPS (%) at various soil depths under different treatments.

Soil depth (cm)	MS[a]–2014				FS				MS-2015			
	C0[b]	CN	P0	PN	C0	CN	P0	PN	C0	CN	P0	PN
10	41.8 a[c]	42.0 a	38.2 b	37.3 c	49.9 a	49.9 a	49.8 a	48.5 a	46.6 a	46.9 a	44.5 b	42.1 c
20	49.3 a	49.0 a	44.2 b	43.1 c	52.3 a	52.6 a	51.7 a	51.5 a	50.5 a	49.8 a	48.0 b	45.1 c
30	57.0 a	56.0 a	51.0 b	50.1 b	58.8 ab	59.5a	58.3 b	58.4 b	57.5 a	57.6 a	54.8 b	50.8 c
40	51.8 a	52.2 a	46.5 b	46.0 b	54.1 a	54.0 a	53.5 a	53.6 a	53.0 a	53.0 a	49.8 b	45.9 c
50	54.1 a	54.8 a	48.3 b	47.6 b	55.8 a	56.2 a	55.4a	55.1 a	55.6 a	54.7 a	51.4 b	47.5 c

[a] MS and FS denote the maize growing season and fallow season, respectively.
[b] Definitions of the codes for the treatments are shown in the footnotes of Table 2.
[c] Mean values (n = 3) followed by different letters within a row in the same seasons are significantly different at P < 0.05.

concentrations then decreased as the soil dried. However, the heavy rainfall (130 mm) event that lasted from 8 to 17 September in 2014 did not increase CO_2 concentrations as appreciably as the event that lasted from 5 to 9 August in 2014.

In contrast to the unplanted treatments, the CO_2 concentrations increased more dramatically in the planted treatments at soil depths from 10 to 50 cm, especially in the deeper layers after early June, when the maize began to joint (Fig. 4). CO_2 concentrations near the ground (0 cm) showed no difference among different treatments (Table 4). The presence of maize plants significantly increased the soil CO_2 concentrations at depths of 10–50 cm in the maize growing period in both years. Compared with the P0 treatment, N fertilization significantly decreased the CO_2 concentrations in the PN treatment at the depths of 10, 20 and 30 cm in 2015 ($P < 0.05$). CO_2 concentrations for the planted soil were somethat higher than those for the bare soil during the fallow season.

4.2.2. CO_2 effluxes with time and depth

Soil CO_2 flux rates at all soil depths increased from early June, reached maximum values around early August (milk stage) and then decreased gradually (Fig. 5). The soil CO_2 flux in the 50 cm soil layer was lower than the fluxes in the other layers. Heavy rainfall events that lasted from 5 to 9 August in 2014 (93 mm), from 13 to 17 September (67 mm) in 2014 and from 8 and 14 August in 2015 (112 mm) sharply reduced CO_2 flux rates, although the CO_2 concentrations dramatically increased (Fig. 4). The CO_2 flux rate from late June to early August in 2014 remained extremely high, mainly due to the high diffusion coefficient values during this soil drying period (Fig. 3), because low soil water content was beneficial to CO_2 gas diffusion from the deep soil to the surface.

To validate the estimated CO_2 effluxes, we used the CO_2 emissions measured using the chamber method (except for the fluxes after heavy rainfall) to compare with the estimated effluxes (Fig. 6). There was a linear relationship between the estimated CO_2 effluxes at a depth of 10 cm and the measured emissions both in the planted and the unplanted treatments, however, CO_2 flux values were severely underestimated using the gradient method and the estimated annual cumulative effluxes of the 2014–2015 season were only approximately 25% of the values obtained by the chamber method (Table 6).

CO_2 fluxes in the unplanted treatments were approximately half of those in the planted treatments during the maize growing season in both years (Table 5). The CO_2 flux rates at depths of 0–50 cm during the maize growing season for the unplanted treatment ranged from 21 to 47 mg CO_2 $m^{-2}h^{-1}$ in 2014 and from 26 to 49 mg CO_2 $m^{-2}h^{-1}$ in 2015. The variation during the maize growing season was relatively stable for the unplanted treatments compared with that observed for the planted treatments. No clear difference was observed during the fallow season.

N addition increased CO_2 fluxes in 2015 in the planted soil, mainly in the 20–50 cm soil layers from late August (R5 stage) to harvest (Fig. 5). The mean CO_2 flux rates during the maize growing period for the P0 treatment were 81, 83, 72, 85 and 53 mg CO_2

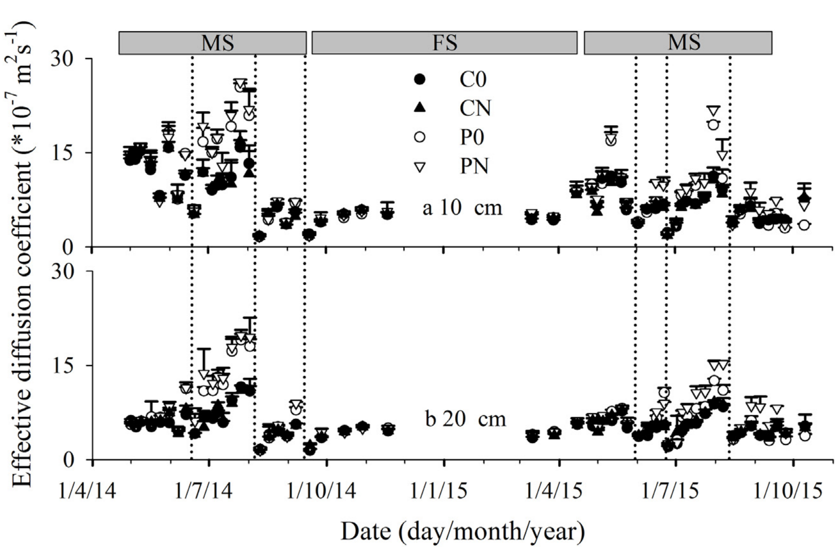

Fig. 3. CO_2 diffusion coefficient (D_p) within soil profile of different treatments during the study period. The bars represent the standard deviations of the means (n = 3). Definitions of the codes for the treatments are shown in the footnotes of Fig. 2.

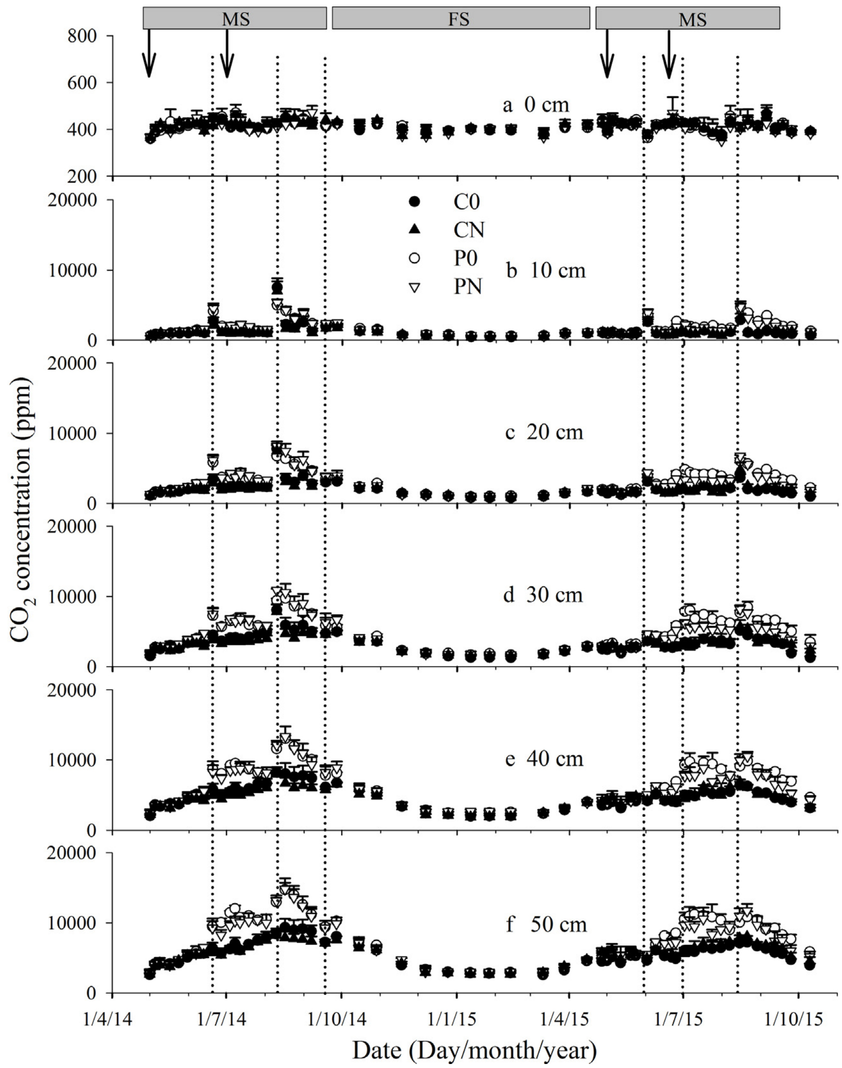

Fig. 4. CO_2 concentration within soil profile under different treatments. The bars represent the standard deviations of the means (n = 3). The solid arrows indicate fertilization, and the dotted lines indicate rainfall. Definitions of the codes for the treatments and seasons are shown in the footnotes of Fig. 2.

$m^{-2} h^{-1}$ in 2014, and were 59, 56, 54, 52 and 38 mg CO_2 $m^{-2} h^{-1}$ in 2015 at the soil depths of 10, 20, 30, 40, and 50 cm (Table 5), respectively. CO_2 fluxes for the PN treatment were significantly higher than those for the P0 treatment in the 30 cm soil layer in 2014 and in the 40 cm layer in 2015. Overall, the cumulative CO_2 fluxes of each layer in the upper 40 cm depths were comparable, which were larger than those of the 50 cm layer (Table 6). The cumulative CO_2 fluxes of the planted soil during the maize growing season were approximately twice as large as those of the unplanted soil. In planted soil, N addition increased the cumulative soil CO_2 fluxes of each layer in the top 30 cm soil layers in 2014 and in the 20–50 cm layers in 2015 to different extents. Overall, N inputs increased the cumulative CO_2 fluxes of the 0–50 cm soil layers by 6% ($P = 0.13$) and 18% ($P < 0.01$) in the planted plots in 2014 and

Table 4
Average seasonal soil CO_2 concentration (ppm) at various soil depths under different treatments.

Soil depth (cm)	MS[a]–2014				FS				MS-2015			
	C0[b]	CN	P0	PN	C0	CN	P0	PN	C0	CN	P0	PN
0	419 a[c]	419 a	418 a	417 a	403 a	402 a	404 a	400 a	418 9 a	414 a	416 a	413 a
10	1663 b	1491 b	2019 a	2172 a	926 bc	830 c	1000 ab	1060 a	1248 c	1178 c	2030 a	1831 b
20	2693 b	2434 b	3603 a	3860 a	1507 b	1532 ab	1657 ab	1721 a	2057 c	2015 c	3519 a	3198 b
30	4194 b	3709 b	5640 a	5932 a	2427 b	2409 b	2674 a	2743 a	3346 c	3319 c	5483 a	4863 b
40	5597 b	4994 b	7518 a	7500 a	3458 bc	3284 b	3736 ab	3896 a	4745 b	4895 b	7073 a	6588 a
50	6464 b	5965 b	8813 a	8637 a	4199 ab	4048 b	4492 ab	4547 a	5766 b	6180 b	8326 a	7949 a

[a] MS and FS denote the maize growing season and fallow season, respectively.
[b] Definitions of the codes for the treatments are shown in the footnotes of Table 2.
[c] Mean values (n = 3) followed by different letters within a row in the same seasons are significantly different at $P < 0.05$.

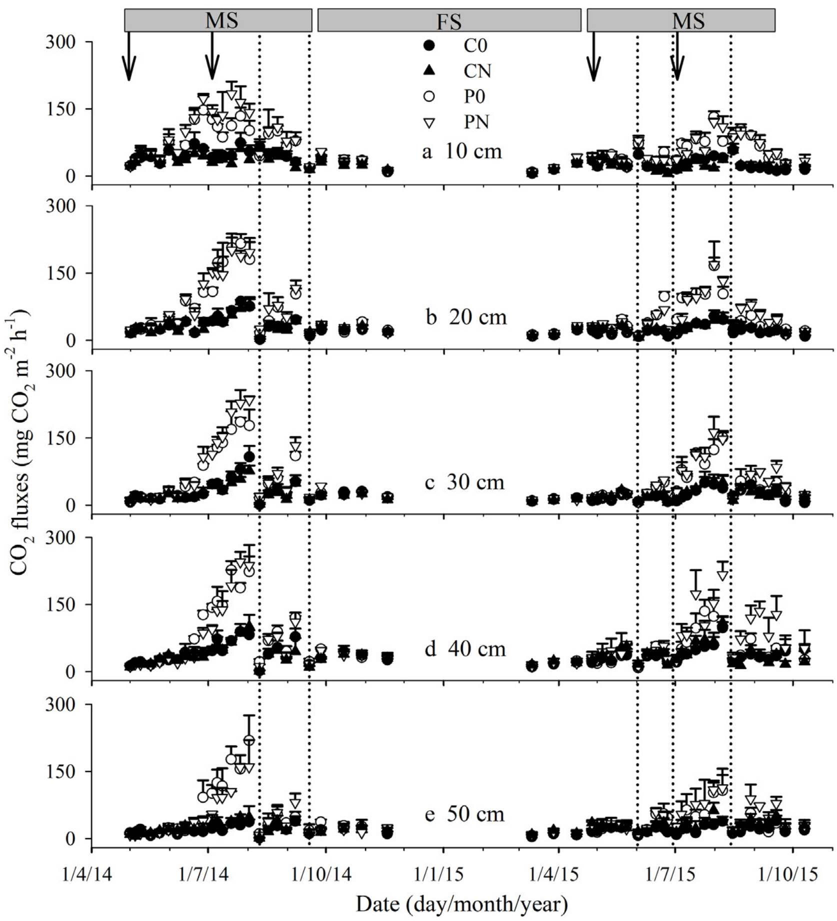

Fig. 5. CO_2 efflux within soil profile under different treatments. The bars represent the standard deviations of the means (n = 3). The solid arrows indicate fertilization, and the dotted lines indicate rainfall. Definitions of the codes for the treatments and seasons are shown in the footnotes of Fig. 2.

2015, respectively. Additionally, N fertilization significantly improved the plant-derived CO_2 fluxes by 20% (P = 0.04) and 29% (P = 0.02) in 2014 and 2015, respectively. In bare soil, N fertilization slightly decreased (P > 0.05) the cumulative CO_2 fluxes of each soil layer (except for the 50 cm) in 2014, but increased the fluxes in respective soil layers located at depths of 20–40 cm (P > 0.05) in 2015. As a result, the cumulative CO_2 fluxes during the maize growing season in the 0–50 cm soil layer were reduced by 9% in 2014 (P = 0.23) and enhanced by 8% (P = 0.21) in 2015 by N fertilization. Cumulative CO_2 fluxes ranged from 599 to 1447 kg CO_2 ha^{-1} among all the treatments during the fallow season, with relatively larger values for the planted treatments. The plant-derived CO_2 fluxes remained low during the fallow season.

4.3. Contribution of maize plant-derived effluxes to soil total effluxes

Before seeding stage (approximately 10 May), the contribution of the plant-derived CO_2 fluxes to the total CO_2 fluxes fluctuated

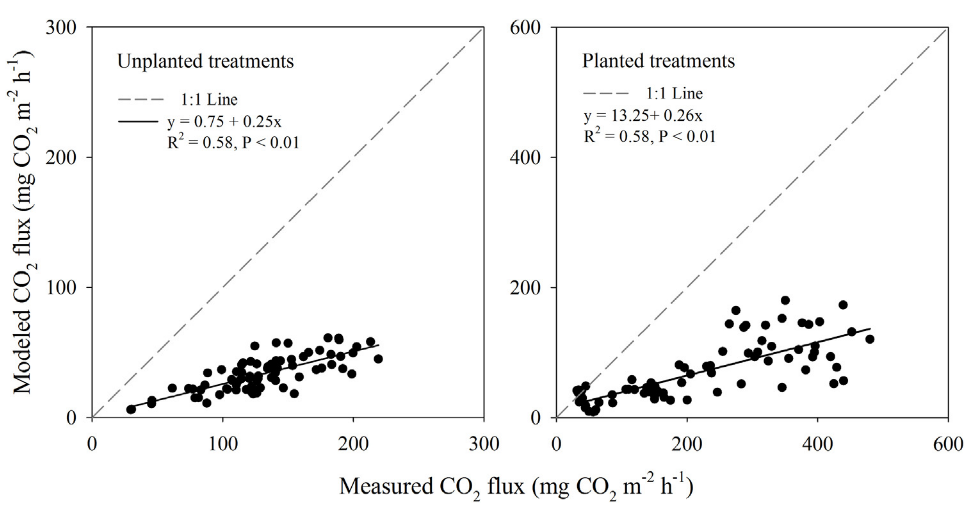

Fig. 6. Comparison of the calculated and chamber measured soil CO_2 effluxes.

Table 5
Average seasonal soil CO_2 fluxes ($mg\,CO_2\,m^{-2}\,h^{-1}$) at various soil depths under different treatments.

Soil depth (cm)	MS[a]–2014				FS				MS-2015			
	C0[b]	CN	P0	PN	C0	CN	P0	PN	C0	CN	P0	PN
10	47 b[c]	40 b	81 a	95 a	24 ab	21 b	26 ab	29 a	32 b	29 b	59 a	59 a
20	38 b	34 b	83 a	88 a	18 b	22 ab	22 ab	25 a	28 b	30 b	56 a	60 a
30	33 c	28c	72 b	80 a	18 ab	16 b	19 a	20 a	30 b	31 b	54 a	54 a
40	41 b	38 b	85 a	78 a	25 a	25 a	28 a	29 a	42 c	49 c	52 b	72 a
50	21 b	23 b	53 a	51 a	14 a	16 a	19 a	18 a	26 c	35 bc	38 ab	48 a

[a] MS and FS denote the maize growing season and fallow season, respectively.
[b] Definitions of the codes for the treatments are shown in the footnotes of Table 2.
[c] Mean values (n = 3) followed by different letters within a row in the same seasons are significantly different at $P < 0.05$.

around 0% at all soil depths (Fig. 7). The ratios of the layers located in the top 20 cm began to increase later due to the growth of roots. However, the ratios of the 30–50 cm layers remained nearly 0% until late May. Thereafter, the maize grew vigorously, and the contributions at various depths remained above 40% from late June to late August. After that period, the ratios tended to decrease as the season progressed. The contributions of plant-derived CO_2 fluxes in the top 40 cm soil layers increased with the addition of N mainly after late August (during milk stage), and the pattern was more evident in 2015. The plant-derived CO_2 effluxes ratios during the maize growing season in the 10, 20, 30, 40, and 50 cm soil layers in 2014 were 0.43, 0.56, 0.53, 0.50 and 0.59 for the P0 treatment and 0.58, 0.61, 0.65, 0.51 and 0.53 for the PN treatment (Table 6), respectively. The ratios presented a general downward trend with the depth of soil profiles in 2015, with values of 0.53, 0.56, 0.49, 0.33, and 0.44 for the P0 treatments and 0.58, 0.57, 0.54, 0.45 and 0.42 for the PN treatment from 10 to 50 cm, respectively. Overall, the ratios of the whole soil profile (0–50 cm) for the P0 treatment were 0.52 in 2014 and 0.47 in 2015, and the application of N fertilizer increased the contribution by 13% ($p = 0.06$) in 2014 and by 9% ($P = 0.13$) in 2015.

5. Aboveground dry matter accumulation

Overall, the application of N fertilizer increased the accumulation of dry matter over the entire growing season in both years (Fig. 8). In 2014, no remarkable difference in dry matter accumulation was observed between the P0 and PN treatments until R3 stage. The accumulation of dry matter accumulation for the P0 treatments increased slowly after R3 stage, but it still maintained rapid growth in the PN treatments in 2015. The total plant-derived fluxes for the 0–50 cm layer during the maize growing season were significantly positively correlated with aboveground dry matter accumulation in both years (Fig. 8).

6. Discussion

6.1. Spatial and temporal variations in carbon dioxide

The large spatial and temporal variations of CO_2 concentrations in the soil profile were governed by a complex interplay of factors regulating soil CO_2 production and transfer through soil layers. Soil CO_2 concentrations increased with soil depth (Fig. 4), commonly

Table 6
Seasonal cumulative CO_2 fluxes ($kg\,CO_2\,ha^{-1}$) of each soil layer under different treatments.

Seasons	Soil depth (cm)	Soil CO_2 fluxes ($kg\,CO_2\,ha^{-1}$)				Plant-derived CO_2 fluxes ($kg\,CO_2\,ha^{-1}$)		Contribution of plant-derived CO_2 fluxes (%)	
		C0[a]	CN	P0	PN	P0 − C0	PN − CN	P0	PN
MS[b]–2014	Surface[c]	5786 ± 434 c	5553 ± 118 c	10168 ± 159 b	11657 ± 657 a				
	10	1588 ± 239 c[d]	1346 ± 255 c	2769 ± 206 b	3251 ± 375 a	1181 ± 133 a	1905 ± 621 a	0.43	0.58
	20	1250 ± 115 b	1147 ± 75 b	2815 ± 113 a	2989 ± 259 a	1565 ± 9 a	1842 ± 294 a	0.56	0.61
	30	1146 ± 121c	965 ± 82 c	2423 ± 107 b	2740 ± 155 a	1277 ± 155 a	1775 ± 230 a	0.53	0.65
	40	1426 ± 250 b	1309 ± 152 b	2856 ± 190 a	2672 ± 177 a	1431 ± 364 a	1363 ± 89 a	0.50	0.51
	50	722 ± 7 b	808 ± 220 b	1781 ± 66 a	1746 ± 303 a	1059 ± 65 a	938 ± 307 a	0.59	0.53
	0–50	6131 ± 667 b	5575 ± 406 b	12644 ± 182 a	13397 ± 711 a	6513 ± 486 b	7822 ± 560 a	0.52	0.58
FS	Surface	3799 ± 101 bc	3680 ± 324 c	4268 ± 101 b	5198 ± 414 a				
	10	892 ± 157 ab	832 ± 176 b	944 ± 45 ab	1083 ± 53 a	52 ± 179 a	250 ± 208 a	0.05	0.23
	20	865 ± 97 b	974 ± 107 ab	1051 ± 24 a	1083 ± 108 a	187 ± 118 a	109 ± 102 a	0.18	0.10
	30	881 ± 36 a	735 ± 17 b	894 ± 9 a	896 ± 66 a	12 ± 45 b	160 ± 49 a	0.01	0.18
	40	1241 ± 130 a	1307 ± 68 a	1302 ± 124 a	1447 ± 227 a	61 ± 16 b	140 ± 159 a	0.05	0.09
	50	599 ± 97 a	759 ± 241 a	769 ± 45 a	914 ± 122 a	170 ± 137 a	154 ± 299 a	0.21	0.15
	0–50	4479 ± 385 b	4608 ± 249 b	4969 ± 139 ab	5422 ± 488 a	481 ± 298 a	814 ± 240 a	0.10	0.15
MS-2015	Surface	5804 ± 426 c	5752 ± 479 c	11373 ± 180 b	12653 ± 724 a				
	10	1009 ± 11 b	901 ± 73 b	2138 ± 152 a	2152 ± 114 a	1129 ± 163 a	1251 ± 67 a	0.53	0.58
	20	861 ± 42 b	915 ± 73 b	1948 ± 131 a	2138 ± 62 a	1087 ± 103 a	1223 ± 127 a	0.56	0.57
	30	900 ± 218 b	904 ± 31 b	1754 ± 152 a	1991 ± 119 a	855 ± 73 a	1087 ± 150 a	0.49	0.54
	40	1273 ± 113 c	1483 ± 167 bc	1906 ± 138 b	2706 ± 235 a	633 ± 182 b	1223 ± 107 a	0.33	0.45
	50	763 ± 189 c	1005 ± 201 bc	1355 ± 304 ab	1763 ± 240 a	592 ± 118 a	759 ± 330 a	0.44	0.42
	0–50	4806 ± 177 c	5207 ± 401 c	9102 ± 288 b	10750 ± 490 a	4296 ± 274 b	5543 ± 489 a	0.47	0.52

[a] Definitions of the codes for the treatments are shown in the footnotes of Table 2.
[b] MS and FS denote the maize growing season and fallow season, respectively.
[c] Surface indicates CO_2 emissions measured using the chamber method.
[d] Mean values (mean ± stand deviation; n = 3) followed by different letters within a row in the same index are significantly different at $P < 0.05$.

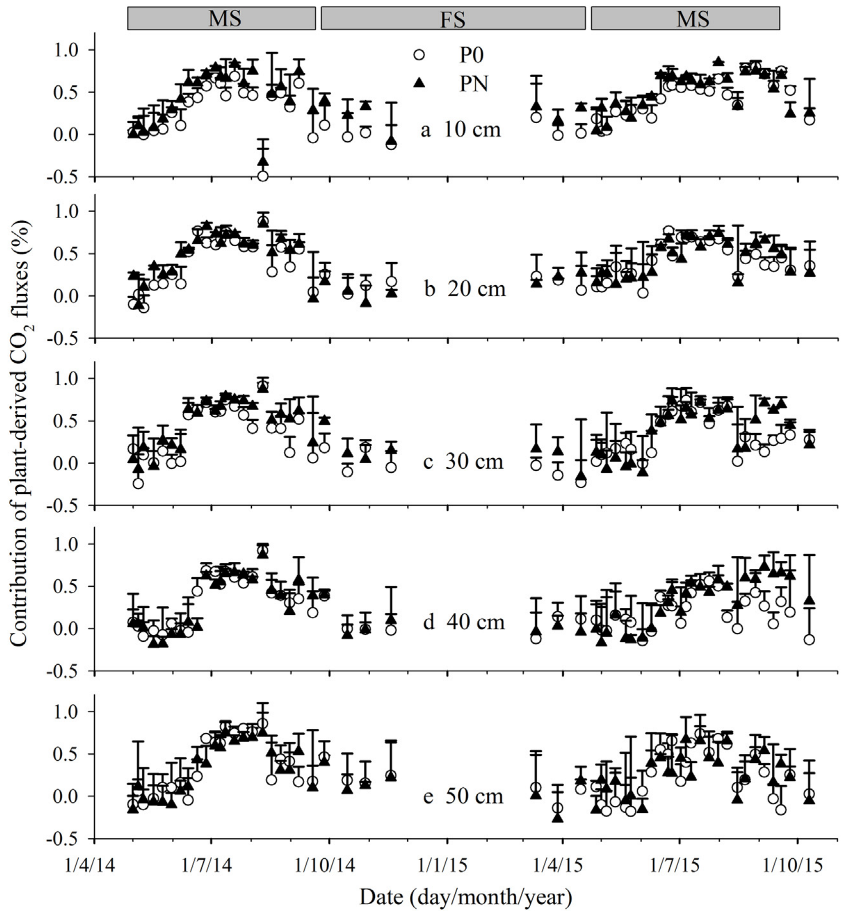

Fig. 7. Contribution of plant-derived CO_2 effluxes to total soil CO_2 fluxes in different soil layers. The bars represent the standard deviations of the means (n = 3). Definitions of the codes for the treatments are shown in the footnotes of Fig. 2.

observed in many studies (Fierer et al., 2005; Pihlatie et al., 2007; Wang et al., 2013; Nan et al., 2016). The seasonal trend in profile CO_2 concentrations was very distinct. Soil CO_2 concentration increased after rainfall events, because water content reduced the diffusion of CO_2 through soil profile and stimulated biological activity (Lee et al., 2004; Fierer et al., 2005; Maier et al., 2011). In contrast, the heavy rainfall event that occurred between 8 and 17 September in 2014 did not induce excessive variation in the CO_2 concentration of each soil layer, which was attributed to the inhibition of CO_2 production. This finding could be explained as follows: First, the rainfall (130 mm) was heavier and the timing (10 consecutive days except for 12 September) was longer (Fig. 2) compared with other rainfall events. Heavy rainfall restricted the soil macro porosity and reduced the soil air-filled pore space such that the supply of oxygen in the soil profile declined; thus, root and microbial respiration were inhibited (Ball et al., 1999), and in turn, CO_2 production was inevitably reduced. Second, the low temperature measured during these rainy days may have also decreased

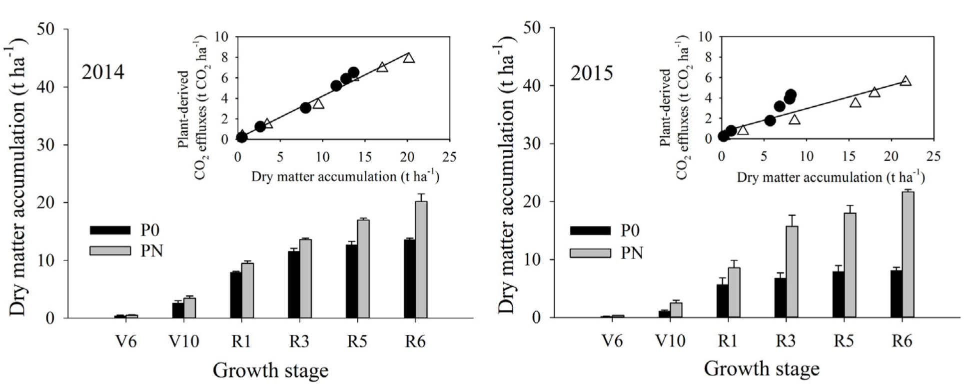

Fig. 8. Aboveground dry matter accumulation under N treatments in 2014 and 2015. The bars represent the standard deviations of the means (n = 3). Dark circles represent P0, and open triangles represent PN. V6, V10, R1, R3, R5 and R6 represent the six-leaf stage, ten-leaf stage, silking stage, milk stage, dent stage and physiological maturity throughout the maize growing period.

the CO_2 production of roots and microorganisms (Harper et al., 2005).

High CO_2 concentrations may not necessarily result in high CO_2 efflux because of a change in diffusivity (Guo et al., 2015). We observed a pronounced increase in CO_2 concentration (Fig. 4) after rainfall, whereas the CO_2 flux largely declined (Fig. 5), particularly on 10 August in 2014, because the fluxes in the soil layer depend not only on the concentration gradient and but also on the diffusion coefficient. Heavy rainfall increased the CO_2 concentration in each soil layer, whereas the concentration difference and diffusion coefficient were largely reduced; as a result, the flux was dramatically reduced.

Some results showed that the uppermost soil layers were responsible for the most of the CO_2 production (Kusa et al., 2008; Pumpanen et al., 2008; Xiao et al., 2015). However, we found that the CO_2 fluxes were approximately the same as the upward fluxes from the 10, 20, 30 and 40 cm layers, which indicated that a relatively large portion of soil respiration took place in the deeper soil layers. This phenomenon could be explained as follows: During the maize growing season, especially from late June to early August, limited precipitation and large evaporation led to lower soil water content. Therefore, biotic CO_2 production in the shallow soil layer decreased, especially for the maize planted soil, because large quantities of soil water were consumed by the vigorously growing plants. In contrast, subsurface layers (bellow 20 cm) retained relatively higher soil water content than that of the surface layers, and produced a comparable amount of CO_2 and then diffused upwards, resulting in a higher CO_2 fluxes in the deeper layers. As a result, the seasonal cumulative CO_2 fluxes of different layers were similar in general. Fierer et al. (2005) found that subsurface layers (below 40 cm) in semiarid grassland soil contributed more in whole-profile CO_2 production during the dry season. Sanderman and Amundson (2010) also found that the surface horizon (0–20 cm) contributed less than 20% of the total CO_2 production when the surface soil was dry, while a much greater proportion of total production was in deeper horizons in grassland ecosystems. It has also been reported that CO_2 effluxes of the 0–30 and 30–60 cm layers had a similar seasonal pattern, and the CO_2 effluxes in the 30–60 cm layer were somewhat higher than those in the 0–30 cm layer in a wheat-summer maize rotation system in North China Plain (Wang et al., 2014). In addition, abiotic production of CO_2 due to carbonate precipitation also contributed to the CO_2 flux in the deeper soil profile, however, this CO_2 efflux was of minor importance compared with the biotic source (Kuzyakov, 2006; Schindlbacher et al., 2015).

The estimated CO_2 effluxes of the 10 cm depth were linearly correlated with the measured surface CO_2 emissions (in the presence or absence of maize plant), whereas the estimated flux values were largely lower. The estimated annual cumulative effluxes of the 2014–2015 season were only approximately 25% of the values obtained using the chamber method. The estimated cumulative fluxes during the maize growing season of the two years averaged 2454 and 2702 kg CO_2 ha^{-1} for the P0 and PN treatments, lower than the values of 2887–3920 kg CO_2 ha^{-1} in the 0–7 cm layer reported by Nan et al. (2016) in a maize field near our sites. The values were lower than the annual cumulative soil respiration of 6600 to 57933 kg CO_2 ha^{-1} yr^{-1} in global croplands (Chen et al., 2010), and also greatly lower than the results (17197–23870 kg C ha^{-1} yr^{-1}) in a three-year study conducted in a maize field near our site (Zhang et al., 2015).

In our study, the D_P values at depths of 10 and 20 cm ranged from 0.2–2.6 $*10^{-6}$ m^{-2} s^{-1}, which were lower than the values (1–7 $*10^{-6}$ m^{-2} s^{-1}) reported by Jassal et al. (2004) and Tang et al. (2005) in forest soils. We inferred the underestimation of the CO_2 fluxes using the gradient method derived from the underestimation of the effective diffusion coefficients. Many empirical models have been widely used to calculate the D_P mainly from soil air-filled porosity and soil porosity (Allaire et al., 2008; Jassal et al., 2005; Maier and Schack-Kirchner, 2014); however, there is no universal best model for a specific soil. It has been reported that difference between the estimated and measured fluxes came from the D_p values (Jassal et al., 2005; Maier and Schack-Kirchner, 2014; Pingintha et al., 2011; Tang et al., 2003). Therefore, we compared the results calculated by three different diffusivity models (see the SI). Although the CO_2 fluxes calculated by different models varied greatly, the estimated contributions of the plant-derived CO_2 fluxes yielded a similar result. The results showed that D_p calculated using different models had a significant effect on the absolute values of CO_2 flux for all the treatments, however, the ratios of the differences between the treatments were affected little generally.

6.2. Soil CO_2 with and without roots

Carbon dioxide (CO_2) is produced in soils mainly as the result of the respiratory activity of plant roots and soil microorganisms. Plants play a critical role in the soil profile CO_2 concentration produced by root and rhizomicrobial respiration (Philippot et al., 2009). Plants also increased the gas transport within soil profile and between soil and atmosphere mainly by decreasing soil WFPS, and therefore increased effective diffusion coefficients. In addition, roots penetrated into the soil, which could decrease soil compaction, build secondary macro-pores and create channels, and therefore contributed to the higher gas diffusion from subsoil to atmosphere (Bohn et al., 2011; Philippot et al., 2009). In our study, soil CO_2 concentrations (Fig. 4) and fluxes (Fig. 5) in the planted treatment at soil depths of 0–50 cm were significantly higher than those in the unplanted treatments during the maize growing season in both years. Plants's phenology also strongly influenced CO_2 fluxes (Cheng et al., 2003; Fierer et al., 2005). Before the jointing stage (early June), no visible CO_2 flux difference existed between the planted and the unplanted soil (Fig. 5). Subsequently, the maize grew vigorously, and the difference expanded sharply, peaking between late July and early August. Thereafter, the difference decreased with plant maturity.

We estimated plant-derived CO_2 flux as the difference between CO_2 flux from planted soil and unplanted soil using the root-exclusion method. However, this method suffers from some inevitable shortcomings. First, this method does not consider the priming effect. The rhizodeposition from live roots, especially available C sources, could greatly increase microbial activity and then accelerate SOM decomposition in the rhizosphere (Kuzyakov, 2002). The CO_2 evolved by the priming effect is often significant in mediating plant-soil interactions (Cheng, 2009). Therefore, the absence of plant roots in the bare soil excluded the SOM-derived CO_2 from priming effect, which underestimated the actual SOM-derived CO_2 from planted soil. Second, without plant consumption of soil water and canopy shading, the soil water content and temperatures of the unplanted soil were higher than those of the planted soil. This is one of the main weaknesses of our study due to the fact that soil water and temperature strongly affect the activities of roots and microbe and the associated CO_2 fluxes (Risk et al., 2002). However, in our study, the CO_2 fluxes were calculated from the model with the parameters of effective diffusion coefficients derived from soil WFPS, which may compensate for the drawbacks of the discrepancy of soil water content between planted and unplanted soil in part. In addition, soil nutrient conditions also differed between the planted and unplanted soil due to plant activities. For example, roots absorbed soil available N and released exudates as C sources, which inevitably affected the soil C and N cycling (Kuzyakov, 2002). As a result, the disadvantages of exclusion technique may cause some bias in seperating the plant-derived and SOM-derived CO_2 fluxes. Nevertheless, the root-exclusion method is an inexpensive and simple way to estimate of root-derived and SOM-derived CO_2, which has been widely used (Ding et al., 2010; Ni et al., 2012; Prolingheuer et al., 2014; Tang et al., 2005; Zhang et al., 2013). It has been reported to yield results similar to those of isotopic approaches (Gavrichkova and Kuzyakov, 2008; Rochette et al., 1999). In future research, gradient methods and isotopic techniques should be combined to partition below-ground CO_2 fluxes more accurately.

We attempted to obtain a rough estimate of the average contribution of subsoil plant-derived effluxes to total effluxes in each soil layer (Fig. 7). In general, the contribution of maize plant-derived effluxes to soil total effluxes dramatically increased from early June (V6 stage), indicating rapid root growth during this period. Furthermore, between planting and the V6 stage, the ratio of the top 20 cm layers appeared to be larger than that in the 40 and 50 cm layers, suggesting that roots were distributed in the shallow layers during this period. The contribution of the plant-derived CO_2 flux generally showed a decreasing trend with increasing depth in 2015, possibly because the root volume was mainly concentrated on the surface soil and decreased with depth (Gao et al., 2014; Guan et al., 2014). The two-year mean plant-derived contribution of the whole soil profile (0–50 cm) during the maize growing season was 0.49 for the P0 treatment and 0.55 for the PN treatment (Table 6). The values were higher than the contributions of plant-derived respiration to soil respiration reported by other researchers. Gong et al. (2012) showed that the contribution of root-derived respiration to total soil respiration was 42.7 to 44.8%, as determined by a pot experiment. Zhang et al. (2013) found that the contribution of plant-derived respiration was 0.29, as determined by a field experiment conducted in the North China Plain. However, our values fell in the range of 10%–>90% reported by Hanson et al. (2000). Additionally, our estimates of the contribution were similar with those reported by other authors (Rochette and Flanagan, 1997; Ding et al., 2010; Ni et al., 2012) who found that the contribution of maize plant-derived respiration was 0.44–0.54. In addition, the ratio may correspond to crop types (Zhang et al., 2013), growth stages (Fu et al., 2002), agricultural management practices (Gong et al., 2012) and research methods (Li et al., 2010).

6.3. Effect of nitrogen fertilization on soil CO_2

Many studies have reported that soil respiration is controlled by biotic factors, such as roots and microbes activities (Kelting et al., 1998), and abiotic factors, such as soil temperature (Davidson et al., 1998), soil water content (Gelfand et al., 2015), and substrate supply (Pang et al., 2015). The application of N fertilizer may therefore affect soil CO_2 by changing these factors. In our study, N addition increased the amount of aboveground dry matter, and correlation analysis showed that plant-derived CO_2 fluxes were significantly correlated with aboveground dry matter (Fig. 7), indicating that plant-derived respiration was strongly correlated with maize growth. Moreover, N addition increased cumulative plant-derived CO_2 fluxes in the top 30 cm soil layers in 2014 and the whole soil profile to a different extent in 2015 (Table 6). As a result, the cumulative CO_2 fluxes in the 0–50 cm soil layers during the maize growing season of the PN treatment were 6% ($P = 0.13$) and 18% ($P < 0.01$) higher than those of the P0 treatment in 2014 and 2015, respectively. The dry matter accumulation for the P0 treatment was significantly higher in 2014 than in 2015 (Fig. 8), mainly because the N inputs were lower than the maize N requirement, resulting in soil N depletion (Berenguer et al., 2009) in 2015. Therefore, the N effect on enhancing maize growth and plant-derived CO_2 fluxes was more pronounced in 2015. Overall, the results indicated that N addition enhanced subsoil CO_2 fluxes by improving plant-derived CO_2 effluxes in semiarid croplands. This view has been supported by other researchers (Jassal et al., 2011; Shao et al., 2014).

Differences in soil CO_2 flux between the P0 and PN treatments mainly occurred during stages R5 to R6 in 2015 (Fig. 5). During this period, the dry matter accumulation of the P0 treatment presented practically no increase, whereas it was significantly improved by N inputs (Fig. 8), implying that N fertilization promoted plant development in the late growth period. It has been reported that N addition could delay root death (Peng et al., 2012) and extend the duration of plant growth (Li et al., 2015). N addition has been reported to promote post-silking N uptake by roots and photosynthesis, and increased production of photosynthates may supply additional C to roots (Chen et al., 2015), thus stimulating roots and microbial respiration. In addition, N inputs increased cumulative CO_2 fluxes mainly at soil depths of 40 cm and 50 cm (Fig. 5), indicating the critical role of roots activities in this zone during the late growth stage. This reasoning is supported by the findings suggesting that the effective and active root layers shift from the surface soil layer during early stages to the subsoil (Wiesler and Horst, 1993; Durieux et al., 1994). As maize matures, the 30–60 cm soil layer becomes the most important root zone for nutrient and water uptake (Oikeh et al., 1999).

In contrast, some results indicate that N fertilization has a negative (Mo et al., 2008; Ramirez et al., 2010) or little effect on soil CO_2 effluxes (Sainju et al., 2010; Koehler et al., 2012; Liu et al., 2015). Bowden et al. (2004) proposed that the application of N fertilizer reduced CO_2 production in forestland due to a decline in microbial activity. Ni et al. (2012) showed that N addition had no marked effect on soil CO_2 flux in a maize field in Northeast China; although the plant-derived CO_2 flux was raised by N input, the increase was counteracted by the decline in native SOC decomposition. Sainju et al. (2010) reported that N fertilization did not affect soil CO_2 fluxes overall, although it had a variable effect on CO_2 emission during their study period in dryland cropland in eastern Montana.

Compared with the P0 treatment, N fertilization slightly reduced CO_2 concentrations in the PN treatment in 2015 (Fig. 4). Similar results were obtained by Nan et al. (2016) and Wang et al. (2013). CO_2 concentration at a given depth did not imply the strength of CO_2 production; rather, CO_2 concentration was determined by the relative strength of production/consumption and transport (Oh et al., 2005). First, the CO_2 flux in each soil layer was somewhat higher in the PN treatment than in the P0 treatment in 2015 (Fig. 5); Second, the PN treatment showed relatively higher diffusion coefficient values (Fig. 3), indicating a higher transport rate within the soil layers and across the soil surface to the environment. As a result, we speculate that despite the higher CO_2 flux of the PN treatment, the relatively higher CO_2 diffusion rates during the maize growing season contributed to a relatively lower CO_2 concentration in the soil profile.

There was no consistent effect of N addition on the soil profile CO_2 fluxes in the unplanted treatments during the maize growing season (Table 6). Howerer, the CO_2 fluxes in the top 10 cm of the CN treatment were somewhat lower than those in the C0 treatment in both years, implying that N fertilization possibly decreased the decomposition of native organic carbon (SOC) of surface soil; similar results have been reported (Ramirez et al., 2010; Yan et al., 2010; Ni et al., 2012). In contrast to the negative relationship between N addition and soil fluxes, it has been suggested that N addition stimulates the decomposition of native soil organic carbon by increasing microbial biomass (Ding et al., 2010; Zhang et al., 2014; Zhou et al., 2014). Moreover, soil acidification due to N fertilization may also cause microbial CO_2 flux to decrease

(Treseder, 2008), but this factor was ruled out because of the short duration of our study (i.e., two years).

The contributions of the plant-derived CO_2 fluxes to the total soil CO_2 fluxes of the top 40 cm layers were also slightly enhanced by N inputs in both years, which mainly occurred from stage R5 (late August) to harvest (Fig. 7), due to a substantial increase in the plant-derived CO_2 flux and the small response of the SOM-derived CO_2 flux to N addition during this period. Our results indicate that the different responses of the plant-derived and SOM-derived CO_2 flux to the N addition treatment contributed to the variations in the plant-derived CO_2 flux ratio.

7. Conclusion

Our study is one of few that has separated the subsoil plant-derived CO_2 flux from the total subsoil CO_2 flux in different soil layers at depths of 0–50 cm using the gradient method. The CO_2 concentration and fluxes during the maize growing season were significantly increased by the presence of maize. The estimated cumulative plant-derived CO_2 efflux for the 0–50 cm layer were significantly correlated with the accumulation of aboveground dry matter during different maize growth stages. The dynamics of the contributions of the plant-derived CO_2 flux to the total soil CO_2 flux in each soil layer corresponded with maize growth. In the unplanted soil, N addition slightly decreased and increased the total cumulative CO_2 fluxes for the 0–50-cm layer in 2014 and 2015 respectively. In the planted soil, N inputs increased the total CO_2 effluxes for the 0–50-cm layer, mainly by stimulating the plant-derived CO_2 effluxes during the late growing season. Moreover, the contributions of the plant-derived CO_2 flux to the total soil CO_2 in each layer of the top 40 cm of soil were also generally improved by N addition.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2016.11.020.

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