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Soil respiration components and their temperature sensitivity under chemical fertilizer and compost application: the role of nitrogen supply and compost substrate quality

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Key Points:

- Autotrophic respiration (Ra) had linear while heterotrophic respiration (Rh) had parabolic response to soil nitrogen supply.
- Impact of compost on Ra and Rh was determined by the substrate quality that controlled decay process and nitrogen release.
- Temperature sensitivity of Ra was increased while that of Rh was decreased by chemical fertilizer and compost application.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1029/2018JG004771

Abstract: Understanding autotrophic (Ra) and heterotrophic (Rh) components of soil respiration (Rs) and their temperature sensitivity (Q_{10}) is critical for predicting soil carbon (C) cycle and its feedback to climate change. In agricultural systems, these processes can be considerably altered by chemical fertilizer and compost application due to changes in nitrogen (N) supply and substrate quality (decomposability). We conducted a field experiment including control, urea and four compost treatments. Ra and Rh were separated using the root exclusion method. Composts were characterized by chemical analyses, ^{13}C solid-state NMR, and lignin monomers. Annual cumulative Ra, along with root biomass, increased with soil mineral N, while Rh was suppressed by excessive N supply. Thus, Ra was stimulated but Rh was decreased by urea alone application. Annual Rh was increased by application of compost, especially that containing most lignin vanillyl and syringyl units, O-alkyl C, di-O-alkyl C, and manganese. However, during the initial period, Rh was most effectively stimulated by the compost containing most carbohydrates, lignin cinnamyl units, phenolic C and calcium. Ra was mediated by N release from compost decomposition, and thus exhibited similar responses to compost quality as Rh. The Rh Q_{10} was reduced while Ra Q_{10} was increased by chemical fertilizer and compost application. Moreover, the Rh Q_{10} negatively related to soil mineral N supply and compost indicators referring to high substrate quality. Overall, our results suggest that N supply and substrate quality played an important role in regulating soil C flux and its response to climate warming.

Keywords: Autotrophic respiration; Chemical fertilizer and compost application; Heterotrophic respiration; Lignin; Q_{10} value; Solid-state ^{13}C -CPMAS NMR

1. Introduction

Soil contains at least twice much carbon (C) as the atmosphere, and thus even a small change in soil organic carbon (SOC) can have remarkable impacts on atmospheric carbon dioxide (CO₂) concentration and climate dynamics (Bond-Lamberty et al., 2018). Annually, human-derived reactive nitrogen (N) input to global land surface is approximately 150 Tg, largely originating from fossil fuel combustion and agricultural fertilization (Schlesinger, 2009). Because the biogeochemical C and N cycles are strongly coupled (Abramoff et al., 2017), this N enrichment can be expected to induce considerable changes in C cycling processes, including soil CO₂ emission, i.e., soil respiration (Rs) (Greaver et al., 2016).

Despite numerous studies, the effects of N fertilization on Rs remain controversial in both magnitude and direction (Janssens et al., 2010; Zhong et al., 2016). Debate about the effect of N fertilization on Rs may be due to the fact that Rs consists of two components: autotrophic respiration (Ra) from live plant roots and associated rhizosphere microorganisms and heterotrophic respiration (Rh) from microbial decomposition of organic matter (OM), which are driven by different mechanisms and respond differently to changing environmental conditions (Phillips et al., 2017; Subke et al., 2006). Ra is tightly coupled to root activity and plant photosynthesis (Hopkins et al., 2013; Savage et al., 2013); while Rh is dependent on soil microbial activity and substrate availability (Allison et al., 2010; Sihi et al., 2016). Increased N supply can stimulate plant productivity and thus Ra (Hopkins et al., 2013); in contrast, the belowground carbon (C) allocation may decrease after high N fertilization, leading to a lower Ra (Högberg et al., 2010). N fertilization may promote bacterial growth, hydrolytic enzyme activity, and labile C turnover; alternatively, may inhibit fungal growth,

oxidative enzyme activity and recalcitrant C degradation, resulting in apparently contradictory effects on Rh (Cusack et al., 2010; Janssens et al., 2010; Xu et al., 2016). Previous studies examining the response of Rs components to N addition mainly focus on natural systems, evidences from agricultural soils which receive greatly more N are relatively lacking.

In agricultural systems, organic amendments are commonly used to maintain soil fertility. It is conceivable that the responses of Ra and Rh to organic amendments mainly depend on OM decomposition that mediates substrate supply and N release. Although climate is the major driver of decomposition at a broad scale, the effect of the OM quality might predominate at a local scale (Bradford et al., 2016). Here, “quality” is defined by the OM decomposability; specifically, “high quality” means fast decomposition rate (chemically labile); while “low quality” means slow decomposition rate (chemically recalcitrant) (Cotrufo et al., 2013). The C/N ratio has been used as a quality indicator for a long time; however, there have been a large number of studies showing that this proxy does not necessarily regulate decomposition as it involves the total rather than the labile organic C (Bonanomi et al., 2013; García-Palacios et al., 2016). In the classical view, initial OM decay is controlled by readily degradable compounds (like carbohydrates, small organic acids, and proteins), while lignin usually accumulates and links to the remaining celluloses and hemicelluloses to form lignin-polysaccharide-complex during the later decay (Berg, 2014; Berg & Matzner, 1997). However, traditional proximate analyses may not accurately identify the compounds governing the decay process because the measured indicators are generally operationally defined and provide little direct structural information, such that may improperly describe the

C quality (McKee et al., 2016). For example, the acid-unhydrolysable fraction, commonly termed as lignin, contains many other substances like waxes, cutins and condensed tannins that are extremely resistant to biodegradation (Preston et al., 2009). Recent studies using compound-specific analysis can provide deeper insights into the quality of OM. For example, lignin monomers measured by cupric oxide (CuO) oxidation have shown that lignin may decay more rapidly than the bulk of OM (Schmidt et al., 2011; Thevenot et al., 2010). Indeed, lignin degradation is a co-metabolic process, and can occur in the presence of available organic C during the initial decay stage (Duboc et al., 2014; Klotzbücher et al., 2011). Additionally, there is increasing evidence for the role of metallic elements, including calcium (Ca), magnesium (Mg), and manganese (Mn) in regulating OM decay (Aponte et al., 2012; García-Palacios et al., 2016; Keiluweit et al., 2015). To date, relationships between compost quality, metallic ions and decomposition remain largely undescribed, which hampers the efforts to better guide the application of compost in agricultural soils.

The temperature sensitivity of R_s , described by the Q_{10} value, has drawn considerable attention in recent decades given that R_s may increase with rising temperature, potentially resulting in a positive feedback to global warming (Bond-Lamberty et al., 2018). It has been demonstrated that the Q_{10} values of R_a and R_h are different and respond differently to nutrient supply (Tu et al., 2013; Yan et al., 2010). However, studies addressing the effects of chemical fertilizer and compost application on the Q_{10} values of R_a and R_h are largely lacking. It is possible that the response of Q_{10} would vary with the traits of the added OM because substrate quality can affect the Q_{10} of decomposition (Frey et al., 2013). Although theory suggests that the decay of recalcitrant compounds requires higher activation energy,

and thus has greater sensitivity to increasing temperature (Davidson & Janssens, 2006), there is no consistent relationship between the OM quality and the temperature response of its decomposition (Conant et al., 2011). This is probably because there are a multiply of factors that may affect the Q_{10} , such as soil moisture condition by regulating the diffusion of oxygen, substrates, and enzymes (Davidson et al., 2012; Sihi et al., 2018a), and substrate protection by mineral association and aggregate occlusion (Castellano et al., 2015; Reynolds et al., 2017). In addition, the controversial findings might also be because the operationally defined indicators used in previous studies did not fully represent OM lability or recalcitrance (Conant et al., 2011; Feng & Simpson, 2008). Solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy has been demonstrated to be a powerful tool to identify the composition of OM at a molecular level (Kögel-Knabner, 2002). With this technique, Erhagen et al. (2013) found the Q_{10} of litter decay increased with increasing readily decomposable O-alkyl C and di-O-alkyl C and decreased with increasing resistant aromatic C and phenolic C, which seemingly contradicted the C quality temperature theory. In addition, recent studies have demonstrated that the Q_{10} of decomposition in relation to C quality could also be modulated by the nutrients supply (Eberwein et al., 2015; Sihi et al., 2018b). Therefore, further research is needed to clarify the influences of substrate quality on the Q_{10} of OM decomposition and soil C fluxes in agriculture systems under fertilization.

Compost application is suggested to enhance the SOC content of the cultivated Mollisols in northeast China which have experienced SOC loss for several decades (Jiang et al., 2014). We hypothesized that the quality of compost controls its decomposition and N release and thus affect the magnitude and Q_{10} of R_a and R_h . In the present study, different types of

compost were compared in a field experiment, and were analyzed by chemical methods and ^{13}C -NMR, and their lignin characteristics were obtained by the CuO oxidation procedure. Soil CO_2 fluxes were monitored over one year using closed chamber method, and R_s was partitioned into R_a and R_h by the root exclusion method. Our main objectives were to assess the responses of R_s , R_h , and R_a and their temperature sensitivities to different fertilization regimes, and to link the responses to the supply of N and quality of applied composts.

2. Material and methods

2.1. Study site

The study site was located at the Hailun National Agro-ecological Experimental Station in Heilongjiang Province, China (47°26'N, 126°38'E, 240 m a.s.l.). This area is characterized by a temperate, semi-humid continental monsoon climate with a short hot summer (from mid-late June to August) and long cold winter (from November to early April). The long-term (1953–2013) mean annual air temperature is 1.9 °C, and the annual precipitation is 556 mm. The mean monthly air temperature ranges from -21.6 °C in January to 21.6 °C in July.

The experimental field was a rainfed cropland planted with maize (*Zea mays* L.). The soil is derived from loamy loess and is classified as Typic Hapludoll in the USDA classification. Prior to the commencement of the experiment, the soil (0–20 cm) had a clay loam texture (8% sand, 72% silt, and 20% clay), a pH value of 5.8, bulk density of 1.0 g cm⁻³, and contained 28.5 g kg⁻¹ total organic C (TOC), 2.2 g kg⁻¹ total N (TN), 2.1 mg N kg⁻¹ ammonium (NH₄⁺), 17.7 mg N kg⁻¹ nitrate (NO₃⁻), and 45.4 mg kg⁻¹ dissolved organic C (DOC).

2.2. Experimental design

Twenty-four plots (each in 4.2 m × 9 m) were established in four blocks in May 2012. Each plot within a block was randomly subjected to one of the six fertilization treatments: no N fertilization as control (CONT), all N as urea (UREA), half N as urea and half as composted cattle manure with rice husk (CMRH), composted herb residue with *Artemisia selengensis* straw (HRAS), spent mushroom compost (SPMU), or composted byproducts from furfural and starch production (BPFS). Composts were obtained from commercial sources. Total N application rate was 200 kg N ha⁻¹. In UREA treatment, urea was split into starter fertilizer and side-dressing with a ratio of 1:1. In the compost treatments, composts were applied at a rate of 100 kg N ha⁻¹ as starter fertilizer and urea as side-dressed at a rate of 100 kg N ha⁻¹. Total amount of applied organic C was 1576, 709, 1477, and 1332 kg C ha⁻¹ in the treatment of CMRH, HRAS, SPMU, and BPFS, respectively.

As a local practice, the field was split into ridges and furrows with a distance of 70 cm. On May 16, 2012, all the starter fertilizers were incorporated in the ridges at a depth of 5–10 cm and covered with soil; subsequently maize seeds were sown at a plant spacing of 25 cm. In each plot, an area of 1.4 m × 2.1 m was left unplanted for Rh measurement. Side-dressing of urea occurred at the maize V6–V8 growth stage on June 27, 2012. Mature maize was harvested on October 2, 2012. All the maize grain and straw were removed from the field and samples were collected and oven-dried at 60 °C to constant mass to calculate the grain yield and straw biomass. The root biomass was estimated based on the maize root-shoot ratio measured by Qiao et al. (2014) at the same site as ours.

2.3. Measurements of soil respiration and environmental variables

The static closed chamber method was employed to measure soil CO₂ fluxes from May 16, 2012 to May 14, 2013. The R_s components were partitioned by the commonly used root exclusion method which was implemented by not sowing the maize crop (Chen et al., 2017, 2018). R_a was determined as the difference between R_s and R_h that was measured from the planted and unplanted area, respectively (Suleau et al., 2011). Soil CO₂ fluxes were measured twice per week during the growing season, weekly after maize harvest until soil freezing, and fortnightly during the freezing period. At each gas flux measurement, four gas samples were drawn from the chamber headspace at 0, 10, 20, and 30 min after closure. Gas sampling was conducted in the morning between 09:00 h and 12:00 h of local time. The CO₂ concentration was analyzed on a gas chromatograph (Agilent 7890, Santa Clara, USA). The CO₂ flux was calculated from a linear regression ($R^2 > 0.90$) of the change in CO₂ concentration during the sampling time. Cumulative emission was calculated by linear interpolation between sampling occasions. Detailed descriptions of chamber design and gas sampling have been given previously (Chen et al., 2017, 2018).

Daily precipitation, air and soil temperature, and photosynthetically active radiation (PAR) were measured at a meteorological station located in the study site with a distance of ~20 m from the experimental plots. On the day of gas sampling, soil temperature at 5 cm was measured with a geothermometer, and volumetric soil water content (SWC) at 5 cm was monitored (except during the freezing period) using a TDR probe in each plot. The SWC during the freezing period was estimated using an exponential equation obtained from Tilston et al. (2010), which described the relationship between the proportion of unfrozen water and

temperature in frozen soil. Our previous field study observed that there were no significant differences in temperature and moisture between the planted and unplanted soils (Ni et al., 2012), thus these variables were only measured in the planted subplot in this study. Soils (0–20 cm) were sampled from each plot weekly after maize sowing until soil freezing and at the end of the experiment, totally in 24 times, to measure soil NH_4^+ , NO_3^- , and DOC. Soil mineral N was extracted with 2M KCl, and colorimetrically quantified using a continuous-flow autoanalyzer (San++, Breda, the Netherlands). Soil DOC was extracted with deionized water, followed by centrifugation and filtration $< 0.45 \mu\text{m}$, and determined with a TOC analyzer (vario TOC Cube, Elementar, Hanau, Germany). For the initial soil samples, texture was analyzed using a laser particle size analyzer (LS13320, Beckman Coulter, Brea, USA); pH was measured in a 1:2.5 soil-to-water ratio; bulk density was determined by the coring method; and the total C and N contents were analyzed on a CN analyzer (Vario Max CN, Elementar, Hanau, Germany).

2.4. Analyses of the chemical properties of composts

Compost TOC, TN, NH_4^+ , NO_3^- , DOC, and pH were measured using similar methods as those for soil. Specific UV absorbance (SUVA) of DOC solution was obtained by normalizing its UV absorbance at 280 nm to the DOC concentration. Readily oxidizable C (ROC) was measured using a modified potassium permanganate oxidation method (Chen et al., 2014). Carbohydrate content was determined by the anthrone colorimetric method (Spohn & Giani, 2010). Proteins were extracted with phosphate buffer and measured using the Bradford method (Roberts & Jones, 2008). Soluble phenols were extracted with 50%

methanol and quantified following the Folin-Ciocalteu method (Austin et al., 2016). Total Ca, Mg, and Mn contents were determined using inductively coupled plasma-atomic emission spectroscopy (IRIS-Advantage, Thermo Jarrell Ash, Franklin, USA). Results of the above compost properties are presented in the Figure S1.

Lignin monomers in composts were analyzed by the alkaline CuO oxidation method (Bahri et al., 2006; Baumann et al., 2013). Sample was oxidized with sodium hydroxide and CuO at 170 °C for 2.5 h in pressure digesters. After centrifugation, acidification, separation with ethyl acetate, derivatization with bis-(trimethylsilyl)-trifluoroacetamide, phenol derivatives were analyzed on a gas chromatograph. Ethyl vanillin was added as internal standard before extraction and *trans*-cinnamic acid before derivatization. Lignin-derived phenols yielded by CuO oxidation consisted of vanillyl units (vanillin, acetovanillone, and vanillic acid), syringyl units (syringaldehyde, acetosyringone, and syringic acid), and cinnamyl units (*p*-coumaric acid and ferulic acid). The sum of those units (VSC) was considered as total lignin. Ratios of cinnamyl to vanillyl units (C/V), syringyl to vanillyl units (S/V), and vanillyl units to VSC (V/VSC) were calculated (Table S1). Generally, higher C/V and S/V, and lower V/VSC indicate greater decomposability of lignin due to greater resistance against degradation of the vanillyl units (Duboc et al., 2014).

Compost organic C composition was characterized by solid-state ¹³C NMR spectroscopy (Avance III 400, Bruker, Fällanden, Switzerland) operating at a frequency of 100.6 MHz. Cross-polarization magic-angle spinning technique with a spinning speed of 14 kHz applied. NMR spectra were obtained with 1 ms of contact time, 0.5 s of recycle delay, and 10 ms of acquisition time. A ramped ¹H-pulse was applied during the contact time to avoid

Hartmann-Hahn mismatches at high spin rotor rates. Relative abundances of seven functional C groups were obtained by integrating signal intensity within their respective chemical shift regions (Figure S2). Four indices were calculated: (1) alkyl/O-alkyl, the ratio of alkyl C to (O-alkyl C + di-O-alkyl C); (2) CC/MC, the ratio of O-alkyl C to methoxyl C; (3) HB/HI, the ratio of hydrophobic C to hydrophilic C; and (4) aromaticity index (AI), the proportion of (aromatic C + phenolic C) to the intensity in 0–160 ppm region (Table S2). High-quality OM (chemically labile with fast decomposition) generally has high CC/MC and low alkyl/O-alkyl, HB/HI, and AI (Bonanomi et al., 2013; Lorenz et al., 2010).

2.5. Data calculation and statistical analyses

Considering the temperature response of Rs, Rh, and Ra may be affected by soil moisture condition (Davidson et al., 2012; Sihi et al., 2018a), a combined exponential and quadratic function was used to calculate the Q_{10} values according to Yan et al. (2010):

$$R = (ae^{bT}) (c\theta^2 + d\theta) \quad (1)$$

$$Q_{10} = e^{10b} \quad (2)$$

where R is the Rs, Rh, or Ra rate ($\text{mg C m}^{-2} \text{ h}^{-1}$); T and θ are soil temperature ($^{\circ}\text{C}$) and volumetric water content (%), respectively; and a , b , c , and d are fitted parameters.

The percentage of compost C mineralized (C_{min} , %) was estimated by (Chen et al., 2018):

$$C_{\text{min}} = [Rh_{\text{compost}} - 0.5 \times (Rh_{\text{UREA}} - Rh_{\text{CONT}}) - Rh_{\text{CONT}}] / C_{\text{compost}} \times 100\% \quad (3)$$

where Rh_{compost} , Rh_{UREA} , and Rh_{CONT} are the cumulative Rh (g C m^{-2}) in the compost treatment, UREA, and CONT, respectively; 0.5 is the ratio of urea-N application rate in the compost treatment to that of the UREA treatment; and C_{compost} is the amount of organic C

input by compost application. This calculation was based on the assumptions that the priming effect induced by OM addition on SOC decomposition is negligible (Johnson et al., 2007; Niknahad-Gharmakher et al., 2012), and that Rh from SOC decomposition was assumed to respond proportionally to N rate at the range of 100–200 kg N ha⁻¹ (Zhong et al., 2016).

Before statistical analysis, data were tested for normality and homogeneity of variance and ln-transformation was used as necessary. One-way analysis of variance with LSD test was implemented to test significant difference with SPSS 18.0 (SPSS Inc., Chicago, USA). A stepwise multiple regression analysis was performed using SPSS to explore the key factors controlling the seasonal variation of Rs, Ra, and Rh fluxes. Linear or exponential regression models were developed by Origin Pro 8.5 (OriginLab, Northampton, USA) to examine the relationships between root biomass, annual Ra and Rh, and the Rh Q_{10} with other variables. Redundancy analysis (RDA) was exerted to test the relationships between cumulative Ra, Rh and C_{\min} and compost properties using the ‘rda’ function in *vegan* package in R version 3.4.4 (The R Foundation for Statistical Computing, Vienna, Austria). The proportion of explained variation was calculated by using adjusted R^2 values. Statistical significance was assessed by the Monte Carlo permutation test with 999 random permutations.

3. Results

3.1. Weather and soil conditions

Total precipitation was 693 mm during the whole experimental period. There was no significant difference in soil water content (SWC) among treatments and thus we presented the mean SWC for all plots in Figure 1a. During the non-frozen period, the measured mean

SWC varied from 15.9% to 54.5%; while, the estimated SWC under sub-zero temperature ranged at 0.6–4.1%. The average air temperature was 1.2 °C. Soil temperature followed the temporal pattern of air temperature except in winter (Figure 1b), ranged from –6.1 to 26.2 °C and averaged at 6.8 °C. Photosynthetically active radiation (PAR) showed similar variation trend to air temperature and tended to decline on rainy days.

The seasonal dynamics of soil NH_4^+ , NO_3^- , and DOC concentrations are illustrated in Figure S3. Annual mean NH_4^+ concentration was significantly increased by N-fertilization (1.5–3.5 vs. 1.3 mg N kg^{-1}). Fertilizer application also significantly enhanced the annual mean NO_3^- concentration from 6.8 mg N kg^{-1} for CONT to 8.8–16.2 mg N kg^{-1} for the compost treatments and further to 25.7 mg N kg^{-1} for UREA. Annual mean DOC concentration was highest in BPFS (65.0 mg C kg^{-1}), followed by SPMU (62.4 mg C kg^{-1}), both of which were significantly higher than other treatments (52.7–60.7 mg C kg^{-1}).

3.2. Soil respiration and its components

The fluxes of R_s , R_h , and R_a gradually increased after the onset of the experiment, maintained relatively high levels from early July to late August, and then progressively declined until maize harvest in October 2012 (Figure 2). The fluxes of R_s and R_a reached maxima in all treatments on July 3, 2012. Maximum R_h flux was observed on 31 July, 2012 in CMRH. It was interesting to note that during the first 2 months, the highest R_h flux among different treatments was measured in BPFS, then in CMRH from mid-July to early September 2012. The fluxes of R_s and R_h were very low during the freezing period, and increased after soil temperature rose above freezing point during the spring thaw period in April 2013.

Annual cumulative Rs was 367 g C m⁻² in the CONT treatment, which was almost the same as that in HRAS (368 g C m⁻²) and UREA (407 g C m⁻²) and significantly lower than those in CMRH, SPMU, and BPFS (Figure 2a). Annual Rh slightly decreased in the UREA treatment but generally increased in the compost treatments in comparison with CONT (Figure 2b). The largest value (268 g C m⁻²) of annual Rh was measured in CMRH, which was similar to that in SPMU and significantly higher than HRAS and BPFS. Annual Ra was generally higher in the N-fertilized treatments (189–215 g C m⁻²) apart from HRAS (146 g C m⁻²), as compared to CONT (163 g C m⁻²) (Figure 2c).

3.3. Factors affecting soil respiration and the Q₁₀

The stepwise multiple regression analysis indicated that soil temperature, followed by SWC and DOC, were the primary drivers for the seasonal trend of the fluxes of Rs and Rh; while, the variation of Ra flux was predominantly controlled by PAR (Table 1). The annual cumulative Rh showed parabolic trend along the annual mean soil mineral N (NH₄⁺ + NO₃⁻) concentrations among different treatments; whereas, annual Ra and root biomass exhibited positive linear responses (Figure 3).

For the compost treatments, the amount of applied organic C did not relate to cumulative Rh during the initial period, but significantly linearly related to cumulative Rh after 3, 5, and 12 months of decomposition (all *P* < 0.05). The RDA analysis indicated that the measured general properties and metal contents (Figure 4a), lignin-derived phenols parameters (Figure 4b), and the characteristics determined by NMR (Figure 4c) could, respectively, explained 98.3%, 98.7%, and 98.6% of the total variations in cumulative Rh. Cumulative Rh over the

first month correlated positively to the compost content of DOC, carbohydrates, proteins, soluble phenols, total Ca, cinnamyl units of lignin, and phenolic C, and ratios of C/V and CC/MC, but reversely to Mg and Mn, V/VSC and methoxyl C. However, all these relationships became weaker from the second month onwards. Meanwhile, cumulative Rh became more closely positively related to C/N, ROC, SUVA, total Mn, vanillyl and syringyl units of lignin, VSC, V/VSC, O-alkyl C, and di-O-alkyl C, and negatively to alkyl C, carbonyl C, and ratios of alkyl/O-alkyl, HB/HI, and AI. Cumulative Ra and Rh among different compost treatments were significantly correlated, except during the initial 2 months (Figure S4). Accordingly, the relationships between cumulative Ra and compost properties were overall similar to those for Rh (Figure S5).

The Q_{10} value of Rs was not significantly changed by chemical fertilizer and compost application, with the exception of SPMU (Figure 5). However, the Q_{10} of Ra and Rh showed discrepant responses. Comparing with CONT, the Q_{10} of Ra was significantly increased, while the Q_{10} of Rh (except CMRH) was significantly decreased by chemical fertilizer and compost application. The Q_{10} of Rh linearly decreased with increasing annual mean soil mineral N concentration across the all treatments (Figure 3c), and was lowest in the UREA and BPFS treatments. In the compost treatments, the Q_{10} of Rh was not related to the amount of applied organic C ($P = 0.52$), but linearly increased with decreasing contents of carbohydrates, proteins, phenolic C and total Ca and increasing V/VSC ratio and total Mn content (Figure 6).

3.4. Plant biomass and compost decomposition

The dry weight of maize grain yield, straw biomass, total aboveground biomass, and estimated root biomass were overall significantly increased by chemical fertilizer and compost application (Table 2). In addition, UREA also exhibited significantly higher grain yield than CMRH and aboveground biomass than CMRH and SPMU.

The percentages of compost C mineralized (C_{\min}) were shown in the Figure 7. BPFS decomposed most rapidly during the initial period, and at 2 months its C_{\min} was 16.3%, significantly higher than other composts (7.8–9.2%). However, the C_{\min} gradually became lowest in BPFS and highest in CMRH from the fourth month onwards. The annual C_{\min} was 48.1% for CMRH, followed by 37.3% for HRAS, 35.1% for SPMU, and lowest for BPFS (26.9%). There were no significant relationships between the amount of applied compost organic C and C_{\min} during different periods of decomposition (all P values > 0.15). The compost general properties, lignin parameters, and NMR characteristics could, respectively, explained 95.0%, 97.5%, and 96.8% of the total variations in C_{\min} , and C_{\min} and Rh generally exhibited similar relationships to compost quality indexes (Figure S6).

4. Discussion

4.1. Responses of soil respiration and its components to N supply

As expected, chemical fertilizer and compost application increased R_s , and the increase was significant in CMRH, SPMU and BPFS (by 14.3–29.0%). However, urea alone application (UREA) did not change R_s . This was mainly due to the decrease in Rh that counteracted the increase in R_a (Figure 2). Similarly, Ni et al. (2012) found that urea

application stimulated Ra, but slightly inhibited Rh, and overall did not significantly alter Rs. Yan et al. (2010) reported that urea addition exerted a significant positive effect on Ra, but a negative one on Rh, resulting in a neutral response of Rs. In the present study, annual Rh was found to initially increase with annual mean soil mineral N concentration, but subsequently decrease when this was above 17.3 mg N kg⁻¹ (Figure 3a). The UREA had highest annual mean soil mineral N concentration (28.9 mg N kg⁻¹) that was above the optimum and thus exhibited a lowest annual Rh among all the treatments.

In line with our results, Bowden et al. (2004) reported that Rh was not changed by low N addition but significantly reduced by high N addition. Enrique et al. (2008) found that low N addition could cause an increase in soil microbial respiration (Rh); on the contrary, high N resulted in a decrease in Rh. Four mechanisms may help to explain this parabolic response of Rh to N supply. Firstly, based on the stoichiometric theory, under low N condition, decomposers would accelerate OM decomposition to acquire N; while, high N supply would suppress this “microbial N mining” (Craine et al., 2007). Secondly, low N addition may increase the activity of hydrolytic enzymes (like glucosidase) and high-quality C decomposition; however, high N can inhibit the activity of oxidative enzymes (like phenol oxidase), low-quality C decomposition and thus Rh (Cusack et al., 2010). Thirdly, microbial growth and biomass can be stimulated by low N addition; on the contrary, excessive N may reduce the abundance of soil microorganisms, especially oligotrophic microorganisms like fungi, leading to a slow decomposition (Fierer et al., 2012). Fourthly, N can react with organic substances to form more chemically recalcitrant compounds, which is also likely responsible for the decrease in Rh under high N supply (Janssens et al., 2010; Xu et al.,

2017).

Different from Rh, Ra increased linearly with soil mineral N, and was highest in UREA. The regression analysis suggested that the dynamics of Ra flux was primarily controlled by PAR (Table 1). It has been demonstrated that Ra is tightly coupled with aboveground photosynthesis and increases with increasing root biomass (Hopkins et al., 2013). The meta-analyses studies generally find that higher N availability can promote plant photosynthesis rate and increase net primary productivity (NPP), resulting in higher root biomass (Greaver et al., 2016). Consistently, we observed that the root biomass increased linearly with soil mineral N (Figure 3). Therefore, the increased root biomass might mainly account for the increase in Ra under higher N supply (Tu et al., 2013). Secondly, the N concentration in maize roots may be significantly increased by N fertilization (Ni et al., 2012), such that the root metabolic rate will increase resulting in higher specific respiration from root growth and maintenance (Burton et al., 2012). Furthermore, higher NPP generally means more rhizodeposition (exudates, secretions, and sloughed cells), which will provide more substrates to rhizosphere microorganisms, leading to higher rhizomicrobial respiration (a part of Ra) (Bowsher et al., 2018). Although the optimum N supply for root growth was larger for microorganism activity as indicated by the different response patterns of Ra and Rh to soil mineral N; it could be expected that excessive N could also inhibit Ra, which necessitates further studies with large gradient of N addition level.

4.2. Regulation of compost substrate quality on soil respiration components

Compared with CONT and UREA, annual Rh was increased by compost amendment

(Figure 2b). The largest annual Rh was observed in CMRH at the end of the experiment, which was partly due to its largest amount of applied organic C. However, during the first 2 months, the largest Rh appeared in BPFS. During this period, Rh and C_{\min} had no significant relationships with the amount of applied organic C, but were closely related to compost contents of DOC, carbohydrates, proteins, soluble phenols, total Ca, cinnamyl units of lignin, and phenolic C, and the ratios of C/V and CC/MC (Figure 4 and S6). All of these parameters were highest in BPFS among the four composts (Tables S1 and S2; Figure S1).

DOC is regarded as the most bioavailable C fraction, and has been proven to be a key controller of early CO₂ fluxes in many incubation studies (Bertrand et al., 2006; Xu et al., 2016), and set as the key substrates for soil microbial utilization in many model studies (Abramoff et al., 2017; Sihi et al., 2016). Carbohydrates and proteins can be easily degraded by a multitude of microbes (Kögel-Knabner, 2002), and thus can largely contribute to initial soil CO₂ fluxes. Consistently, the CC/MC, i.e., ratios of carbohydrate to methoxyl C, also positively related to initial Rh. In contrast, lignin is relatively resistant against biodegradation which can be undertaken only by a limited species of soil microbes (predominantly fungi) due to its recalcitrant polyphenolic structure (Austin et al., 2016). Therefore, it is traditionally believed to be not or rarely degraded in initial decomposition phases (Berg & Matzner, 1997). However, we found that soluble phenols, cinnamyl-type lignin, and phenolic C (represents polyphenols and lignin) had significant positive relationships with Rh during the first 2 months. Similarly, Klotzbücher et al. (2011) observed a sharp decrease of lignin content in decomposing litter after ~80 days of decomposition. In fact, lignin is usually degraded via co-metabolism in the presence of enough available C because the production of ligninolytic

enzymes is highly energy and C demanding (Keiluweit et al., 2015; Klotzbücher et al., 2011). Hence, the decomposition of lignin, mainly the most easily degraded cinnamyl units (Bahri et al., 2006; Baumann et al., 2013), was likely to have occurred during the initial 2 months and made contribution to Rh flux, especially in BPFS. In addition, it was interesting to note that BPFS had highest level of Ca, which has been suggested to facilitate lignin degradation in early decay stage (Aponte et al., 2012).

As decomposition proceeded, labile C substrates were gradually consumed and Rh became more positively related to vanillyl and syringyl units of lignin, O-alkyl C, and di-O-alkyl C which were all highest in CMRH that exhibited the largest C_{\min} , cumulative Rh and Ra in the later period. Vanillyl and syringyl units are more complex and embedded deeper in the lignin structure compared with cinnamyl units; so their biodegradation are slower at higher energy cost (Bahri et al., 2006). The O-alkyl C and di-O-alkyl C are mostly associated with cellulose and hemicelluloses constituents, and their depolymerization could produce available C for lignolytic microorganisms when the available C resources were less than the initial stage (Klotzbücher et al., 2011). On the other hand, the structural polysaccharides (cellulose and hemicelluloses) generally cross-link with lignin, especially vanillyl and syringyl units, to form lignin-polysaccharide complexes (Bahri et al., 2006; Bertrand et al., 2006). Accordingly, it is conjectured that these compounds were degraded simultaneously, contributing to the largest C_{\min} and Rh in CMRH. Additionally, the highest Mn content in CMRH might promote lignin decomposition. Mn has been shown to play an important role in regulating lignin degradation, particularly in the later stage (Aponte et al., 2012). The white-rot fungi have the capacity to open the aromatic ring structure and thus

completely mineralize lignin into CO₂; while brown-rot fungi can only remove the side methoxyl chains from the aromatic ring and thus partially degrade lignin (Berg, 2014). This is because white- but not brown-rot fungi can produce the ligninolytic enzyme of Mn-peroxidase whose activity is highly related to the supply of Mn (Berg, 2014). Furthermore, CMRH had lowest proportions of alkyl C and carbonyl C and ratios of alkyl/O-alkyl and HB/HI that negatively related to Rh during the later and whole experimental period (Figure 4c). Alkyl C, most likely existing in aliphatic compounds, is regarded as particularly recalcitrant form of organic C due to its long-chain structure and high hydrophobicity preventing the access of enzymes (Feng et al., 2010; von Lützow et al., 2006). The carbonyl C is mainly a constituent of aliphatic amide and has been reported to be negatively related to CO₂ emission (Wang et al., 2004).

The Ra exhibited consistent trend among different compost treatments to that of Rh (Figure S4) and similar relationships with compost substrate quality (Figure S5) during the 3–5 months. Annual Ra was found to be lowest in HRAS which also had lower Rh in comparison to other compost treatments (Figure 2). Our previous studies (Chen et al., 2017 & 2018) found that in contrast to Rh, Ra was more dominantly controlled by mineral N than C supply. Consistently, in the current study, the change was more significant in Ra than Rh by the change in mineral N supply in the UREA treatment, while the opposite was true for the treatments receiving compost with various qualities (Figure 2). What's more, as discussed above, Ra was linearly increased with increasing soil mineral N concentration as a result of larger root biomass (Figure 3). Thus, the least N released from compost decomposition, as indicated by its lowest annual mean soil mineral N concentration, presumably leading to the

lowest Ra in HRAS among the compost treatments. Mineralization of N and C are generally coupled in decomposing OM (Abramoff et al., 2017). Therefore, the regulation of compost application on Ra mainly depended on N release from the decomposition process which was controlled by the substrate quality as discussed above.

4.3. Effects of N supply and compost substrate quality on the Q_{10}

Generally, chemical fertilizer and compost application did not affect the Q_{10} value of Rs (Figure 5). Yan et al. (2018) also reported that N fertilization did not alter the Q_{10} of Rs as a result of unchanged Q_{10} of both Ra and Rh in larch plantations. In the present study, however, the Q_{10} of Ra and Rh exhibited positive and negative response, respectively, to chemical fertilizer and compost application, which might cancel each other out. As discussed above, Ra is tightly coupled with plant productivity. Therefore, increased photosynthesis rate and root activity under N fertilization might magnify the positive response of Ra to increasing temperature (Xiong et al., 2018; Yan et al., 2010).

On the contrary, the Q_{10} of Rh decreased with increasing soil mineral N (Figure 3c). The UREA and BPFS had higher mineral N concentration than other treatments, and thus the lowest Q_{10} of Rh. Similarly, Tu et al. (2013) reported that N addition significantly decreased Q_{10} of Rh in a bamboo ecosystem. Mo et al. (2008) attributed lower Q_{10} of Rh under higher N supply to the reduced total soil microbial activity (indicated by lower Rh). In this study, however, Rh was inhibited by N fertilization only in the UREA treatment, but increased in other treatments (Figure 2b). Therefore, we deduced that the negative N effect on the Q_{10} of Rh was more likely attributable to the inhibition of higher N supply on the decomposition of

more recalcitrant C pool which generally needs higher activation energy (Cusack et al., 2010).

In the compost treatments, the lowest Q_{10} value of Rh was present in BPFS. The Q_{10} of Rh may not be altered by the organic C quantity as observed here and by Meyer et al. (2018), but depends on C quality, with positive, negative and non-significant relationships all being reported in the literature (Karhu et al., 2010). We found that the Q_{10} of Rh increased with decreasing carbohydrates, proteins, and phenolic C, and increasing V/VSC (Figure 6). Carbohydrates and proteins are readily decomposed substrates and thus have lower activation energy (Davidson & Janssens, 2006). The vanillyl units are the most recalcitrant lignin monomers (Thevenot et al., 2010). Accordingly, the Q_{10} value of Rh was observed to positively relate to V/VSC. Similarly, Feng & Simpson (2008) found a higher Q_{10} value of vanillyl than other lignin units in a laboratory study. Thus, the highest carbohydrates and protein contents and lowest V/VSC in BPFS might contribute to its lowest Q_{10} . The negative relationship between Q_{10} with phenolic C was seemingly inconsistent with the kinetic theory considering this component was traditionally regarded as low-quality substrate. However, as discussed above, the phenolic compounds can be degraded when the C availability was high during the initial period. Erhagen et al. (2013) also observed a negative response of the Q_{10} of litter decomposition to increasing amount of phenolic C. Thus, the temperature response may be complicated by the fact that the recalcitrant compounds are degraded in co-metabolism with easily degradable energy-rich substrates (Duboc et al., 2014; Klotzbücher et al., 2011; Wilhelm et al., 2019), meaning that the Q_{10} of decomposition process depends on several interactive reactions with distinct temperature sensitivity. Interestingly, we also found that the

Q_{10} could be negatively and positively regulated by Ca and Mn (Figure 6), respectively, which contributed to lignin decomposition in the initial and later stage (Aponte et al., 2012). Therefore, apart from the organic composition, the concentration of Ca and Mn in OM may be also need to track as they may stimulate decomposition of lignin-rich compounds and thus regulate the temperature response of OM decomposition.

Although significantly related to the annual Rh, C/N, ROC, alkyl C, O-alkyl C, and di-O-alkyl C did not relate to the annual C_{\min} and Q_{10} of Rh. Some studies have pointed out that the C/N ratio may not well indicate the OM quality (e.g., Bonanomi et al., 2013). Accordingly, Wagai et al. (2013) also observed the C/N of SOM was not related to the Q_{10} . ROC generally represents a labile C fraction, and Xu et al. (2012) found that the soil with lower ROC exhibited higher Q_{10} of decomposition. However, the ROC pool may also include some complex compounds, like aromatic and aliphatic molecules, that are resistant to degradation (Romero et al., 2018). In line with our findings, Erhagen et al. (2013) reported that the Q_{10} of Rh was unrelated to alkyl C, O-alkyl C, and di-O-alkyl C that usually were robust proxies of C quality. Therefore, we suggest using a multitude of methods concurrently to capture the full ranges of C quality indices so as to help to reconcile the apparently contradictory observations on the impacts of C quality on Q_{10} . What's more, it should be noted that the relationship between the Q_{10} and C quality may be entangled by the N-regulation as found in this study. Under high N supply, the N-inhibition on recalcitrant C decomposition may enable the low-quality OM (with more recalcitrant C) fail to have higher Q_{10} . Therefore, the effect of N supply should be taken into account when addressing the temperature response of the decomposition of OM with various qualities, especially

considering the increase in reactive N in many ecosystems (Greaver et al., 2016).

4.4. Methodological uncertainties

The root exclusion method is a widely accepted method to partition R_s components (Phillips et al., 2017; Subke et al., 2006). In the present study, this method was achieved by not sowing the maize crop, which might generate fewer biases in comparison to root trenching in forests due to less disturbance and, especially the absence of biases induced by severed roots decomposition (Suleaua et al., 2011). It has been demonstrated that the R_a of maize plant obtained as the difference of CO_2 flux from planted and unplanted soils agreed well with those obtained using the natural ^{13}C abundance method in the field (Rochette et al., 1999) and ^{14}C labeling approach under laboratory condition (Gavrishkova & Kuzyakov, 2008). However, our method may still introduce some biases by the likely changes in soil temperature and moisture (Subke et al., 2006). Previous field experiment conducted in the same area by Ni et al. (2012) found that there were only slight increases in soil temperature and moisture in the unplanted than maize-planted treatments. However, these changes may still result in an overestimation of R_h . Future studies should account for this bias and quantify it with the protocol developed by Savage et al (2018). On the other hand, the neglect of rhizosphere priming effect on native SOC decomposition could cause an underestimation of R_h , although this effect might be very minor for maize (Kuzyakov & Cheng, 2004).

The R_h measured in the compost treatments was a mix of CO_2 fluxes from the decomposition of native SOC and applied compost. OM input may result in priming effect on SOC decomposition (Fontaine et al., 2003), although this might be a small fraction of newly

added C on a one-year scale (Liang et al., 2018). The observed relationships between Rh and its Q_{10} with the compost substrate quality may have uncertainties due to the existence of priming effect and the difference in organic C application rate. Thus, there is a need for studies using various types of ^{13}C -labelled OM applied at the same C amount to further investigate the effect of substrate quality on CO_2 emission from the decomposition of SOC and/or added OM and their Q_{10} values.

5. Conclusions

This study demonstrated that the effects of chemical fertilizer and compost application on Rs were complicated by different responses of Ra and Rh. The Ra and Rh exhibited linear and parabolic response to increasing soil mineral N, respectively. Accordingly, urea-N fertilization led to increase in Ra but decrease in Rh. Impact of compost on Rh was determined by the decomposition process that was controlled by the substrate quality. Initial decomposition and Rh was associated with the easily degradable compounds, labile units of lignin and calcium. During the later stage, however, Rh was mainly related to cellulose, stable units of lignin, and manganese. Ra showed similar response to compost as that of Rh, because the substrate quality determined N release from compost decomposition and thus Ra. The dynamics of Ra was predominantly controlled by photosynthesis and its Q_{10} was increased by N fertilization. In contrast, the Q_{10} of Rh was reduced by N fertilization and application of compost especially that containing more constitutes responsible for initial decomposition. These findings suggested that Rs and its components and their Q_{10} were largely regulated by N supply and substrate quality. Incorporating the coupling of OM

decomposition and the cycles of N and some metal elements into C cycle models can help to achieve more accurate predictions of soil C fluxes under future warming climate. This study also implied that combined application of mineral N fertilizer with compost that contains more aliphatic and aromatic fractions, less labile compounds (especially dissolved carbohydrates) and less calcium and manganese, would potentially promote greater C retention in agricultural soils, especially under a warming climate.

Acknowledgements

We gratefully acknowledge the financial support by the Natural Science Foundation of Jiangsu Province, China (BK20171105), the National Natural Science Foundation of China (41701297, 41730753 and 31561143011), and the Chinese Academy of Sciences (XDB15020100). We thank the staff at Hailun National Agro-ecological Experimental Station, Chinese Academy of Sciences for their logistic support and helpful assistance in the field experiment. We would also like to extend sincere thanks to the Editor and reviewers for their valuable and constructive comments and suggestions that significantly improved the quality of this paper. The authors declare no conflict of interest. Supplementary data including Figure S1–S6 and Table S1–S2 can be found in the supporting information file of this article. All the data used in this study are given in detailed as Table S3–S12 in the supporting information file.

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Table 1. Models fitting soil total (R_s), heterotrophic (R_h), and autotrophic (R_a) respiration against photosynthetically active radiation (PAR), soil temperature (ST), soil water content (SWC), ammonium (NH_4^+), nitrate (NO_3^-), or dissolved organic carbon (DOC) concentration.

Treatment	Equation	R^2
CONT	$\text{Ln } R_s = 0.129 \text{ ST} + 0.047 \text{ DOC} - 0.874$	0.77**
UREA	$\text{Ln } R_s = 0.048 \text{ DOC} + 1.37$	0.47**
CMRH	$\text{Ln } R_s = 0.131 \text{ ST} + 1.73$	0.56**
HRAS	$\text{Ln } R_s = 0.091 \text{ ST} + 2.29$	0.45**
SPMU	$\text{Ln } R_s = 0.032 \text{ DOC} + 2.40$	0.39**
BPFS	$\text{Ln } R_s = 0.093 \text{ ST} + 2.44$	0.43**
All	$\text{Ln } R_s = 0.102 \text{ ST} + 0.034 \text{ SWC} + 0.016 \text{ DOC} + 0.060$	0.61**
CONT	$\text{Ln } R_h = 0.108 \text{ ST} + 0.036 \text{ DOC} - 0.439$	0.74**
UREA	$\text{Ln } R_h = 0.100 \text{ ST} + 0.039 \text{ SWC} + 0.314$	0.86**
CMRH	$\text{Ln } R_h = 0.133 \text{ ST} + 0.065 \text{ SWC} - 0.842$	0.81**
HRAS	$\text{Ln } R_h = 0.058 \text{ ST} + 0.115 \text{ SWC} - 0.466$	0.82**
SPMU	$\text{Ln } R_h = 0.101 \text{ ST} + 0.433 \text{ SWC} + 0.355$	0.74**
BPFS	$\text{Ln } R_h = 0.113 \text{ ST} + 1.54$	0.84**
All	$\text{Ln } R_h = 0.110 \text{ ST} + 0.0424 \text{ SWC} + 0.0106 \text{ DOC} - 0.472$	0.77**
CONT	$\text{Ln } R_a = 0.080 \text{ PAR} + 0.065 \text{ DOC} - 2.42$	0.74**
UREA	$\text{Ln } R_a = 0.047 \text{ PAR} + 2.35$	0.29**
CMRH	$\text{Ln } R_a = 0.100 \text{ PAR} - 0.065 \text{ NO}_3^- + 1.35$	0.72**
HRAS	$\text{Ln } R_a = 0.042 \text{ PAR} + 2.13$	0.34**
SPMU	$\text{Ln } R_a = 0.049 \text{ PAR} + 2.19$	0.35**
BPFS	$\text{Ln } R_a = 0.057 \text{ PAR} + 1.94$	0.48**
All	$\text{Ln } R_a = 0.108 \text{ PAR} + 0.046 \text{ SWC} - 0.115 \text{ ST} + 1.31$	0.45**

Note. * $P < 0.05$, ** $P < 0.01$.

Table 2. Dry mass of maize grain, straw, and root (kg ha^{-1}).

Treatment	Grain	Straw	Aboveground biomass	Root
CONT	8485 \pm 953 c	7827 \pm 748 c	16312 \pm 1699 c	1117 \pm 116 b
UREA	11874 \pm 387 a	11029 \pm 382 a	22903 \pm 746 a	1624 \pm 53 a
CMRH	10028 \pm 274 b	9837 \pm 483 ab	19865 \pm 744 b	1526 \pm 57 a
HRAS	10830 \pm 261 ab	9096 \pm 219 bc	19927 \pm 405 ab	1530 \pm 31 a
SPMU	10685 \pm 311 ab	8537 \pm 469 bc	19223 \pm 749 bc	1476 \pm 58 a
BPFS	10618 \pm 434 ab	9412 \pm 768 ab	20030 \pm 1189 ab	1539 \pm 91 a

Note. Values are shown as mean \pm standard error ($n = 4$). Different letters within the same column indicate significant differences at $P < 0.05$.

Root biomass was estimated according to the maize shoot-to-root ratio measured in the same study site as ours by Qiao et al. (2013).

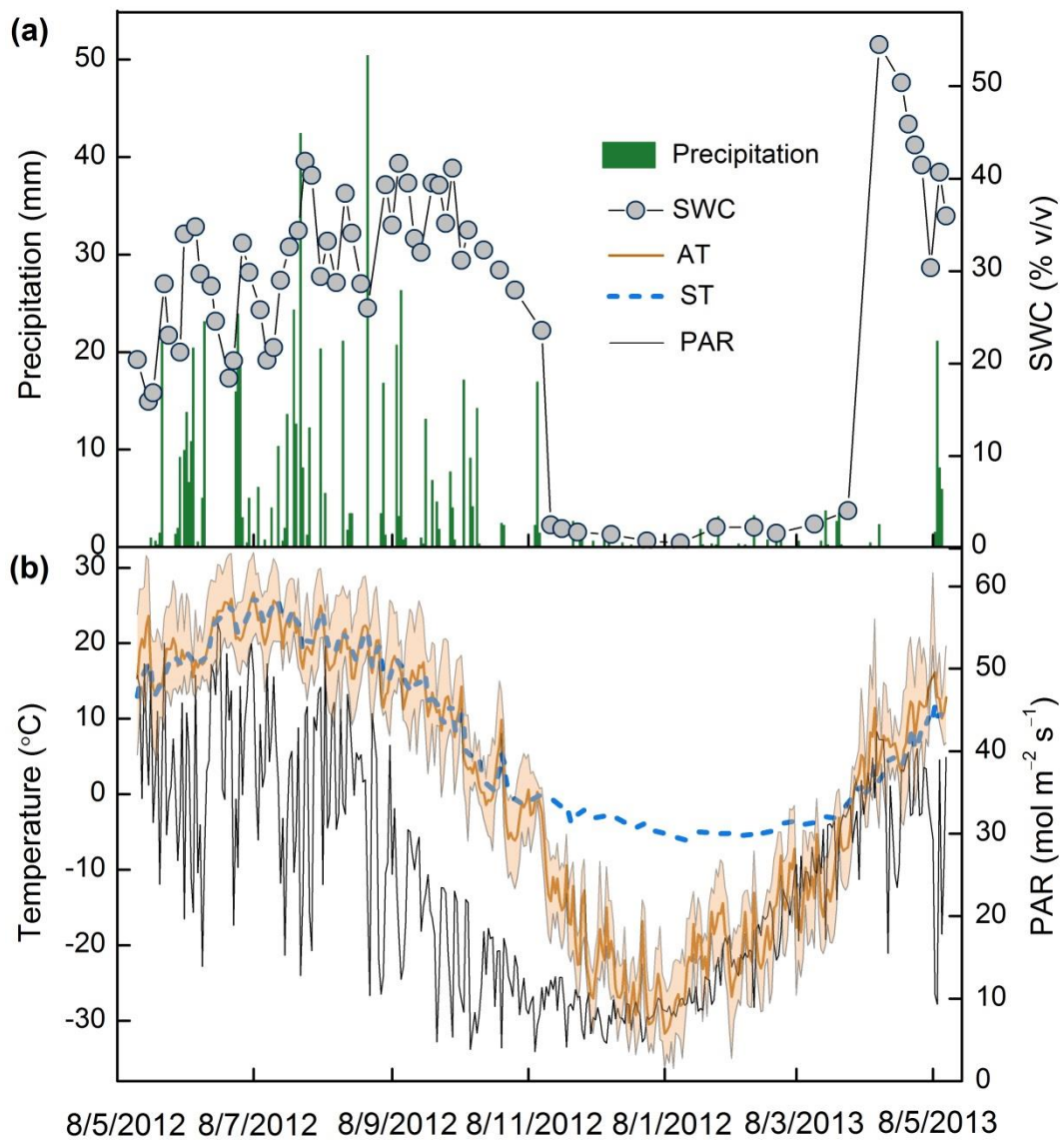


Figure 1. Precipitation, soil (0–5 cm) water content (SWC) (a), air temperature (AT), soil (0–5 cm) temperature (ST), and photosynthetically active radiation (PAR) (b). The shading area in panel (b) indicates the range of daily AT.

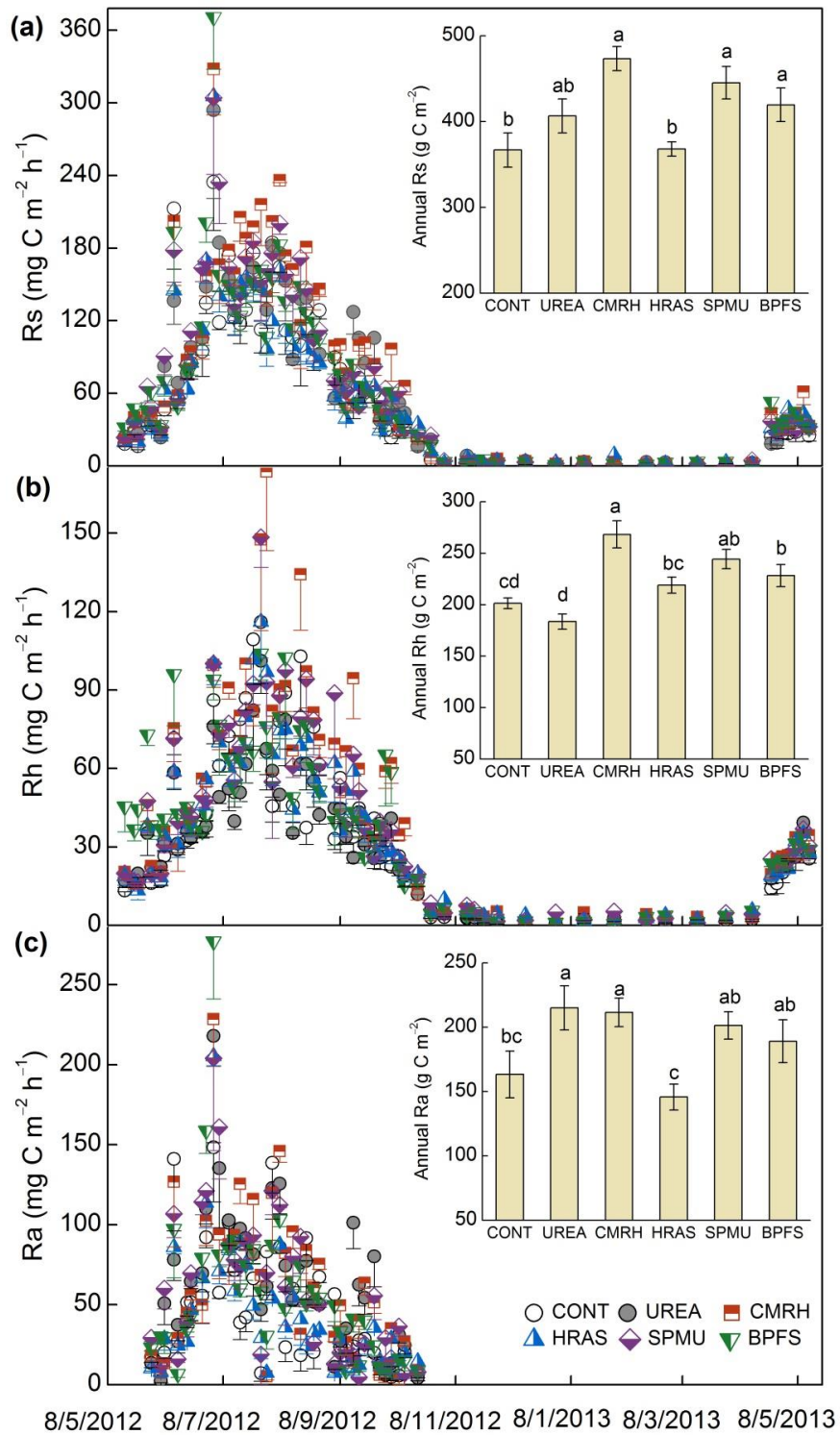


Figure 2. Seasonal dynamics and annual emission of soil respiration (Rs, a), heterotrophic (Rh, b), and autotrophic respiration (Ra, c) in different fertilization treatments. Vertical bars denote standard errors ($n = 4$). Different letters indicate significant differences at $P < 0.05$.

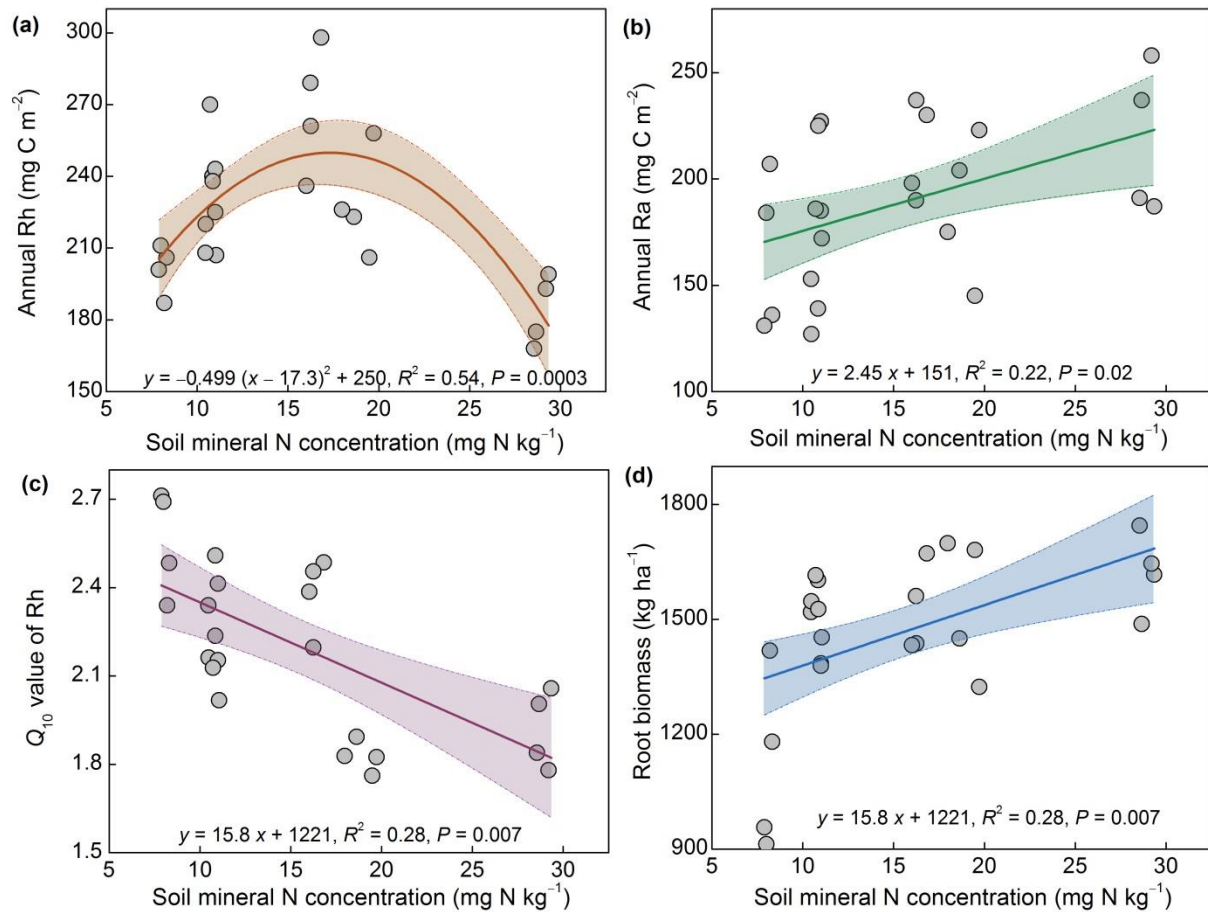


Figure 3. Relationships between annual heterotrophic respiration (Rh; a), autotrophic respiration (Ra; b), Q_{10} values of Rh (c), and root biomass (d) with annual mean soil mineral nitrogen concentration. Shaded areas indicate the 95% confidence intervals.

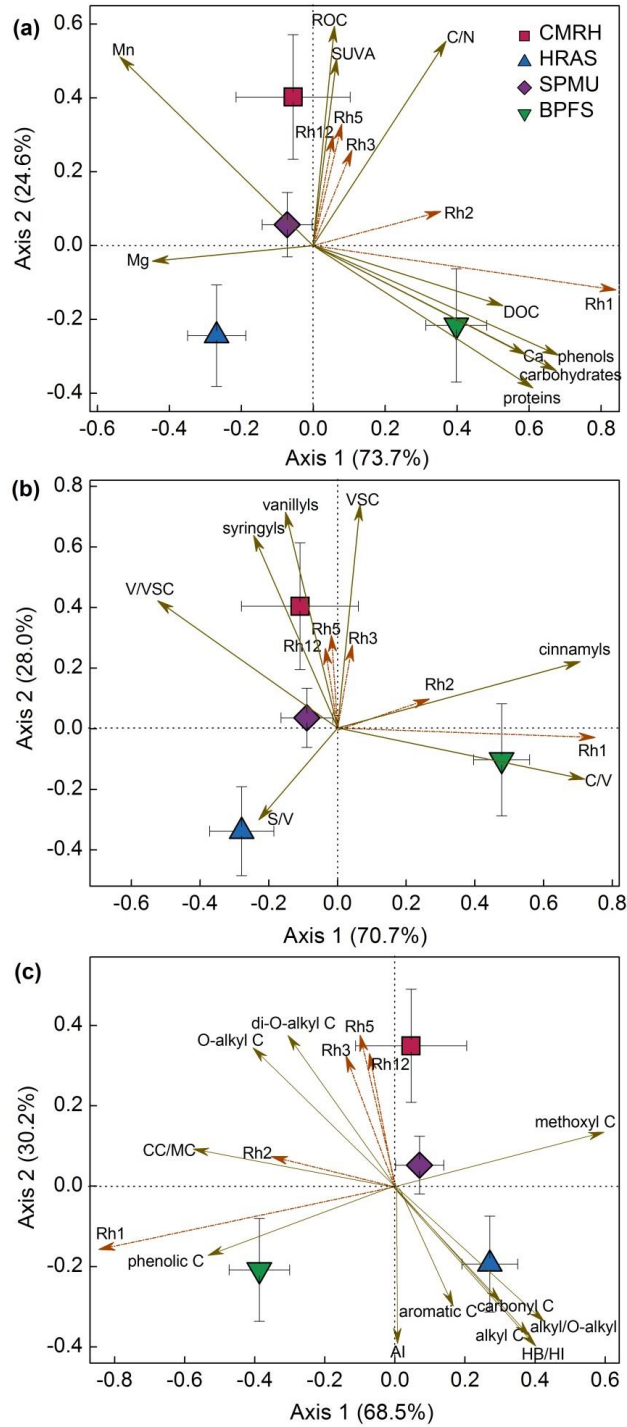


Figure 4. Redundancy analysis (RDA) diagrams showing the relationships between cumulative heterotrophic respiration (Rh) during the period from 0 to 1, 2, 3, 5, and 12 months in the compost treatments and general properties of compost composition and metal contents (a), lignin parameters (b), and characteristics determined by NMR (c).

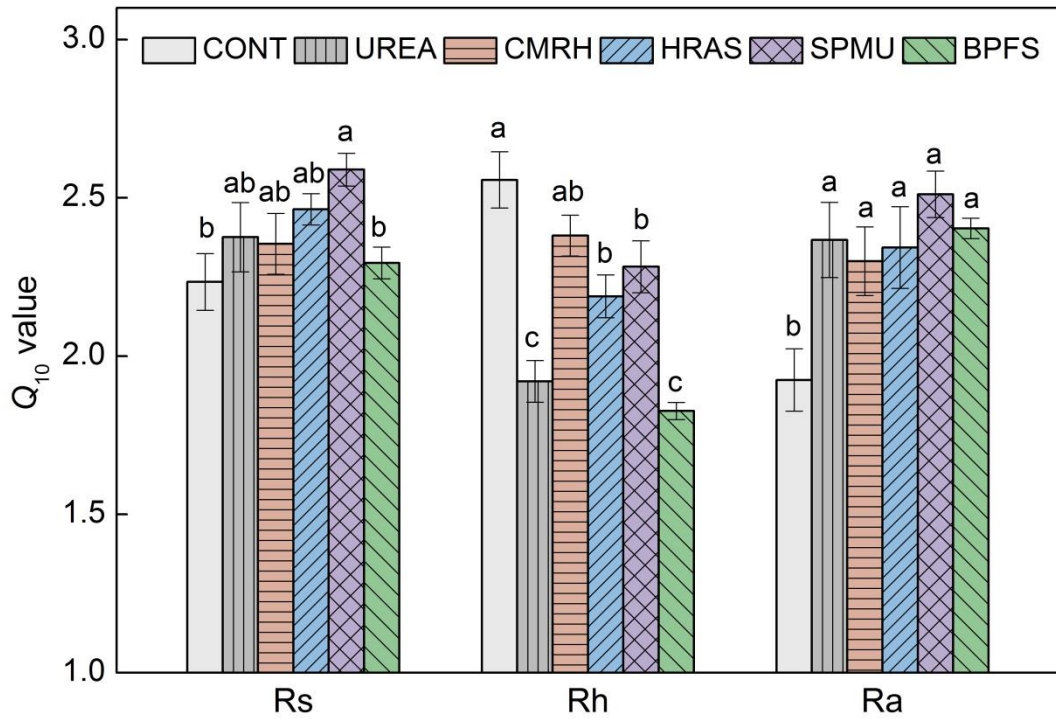


Figure 5. The Q_{10} values of total soil respiration (Rs), heterotrophic (Rh) and autotrophic respiration (Ra) in different fertilization treatments. Vertical bars denote standard errors (n = 4). Different letters indicate significant differences at $P < 0.05$.

Figure 6

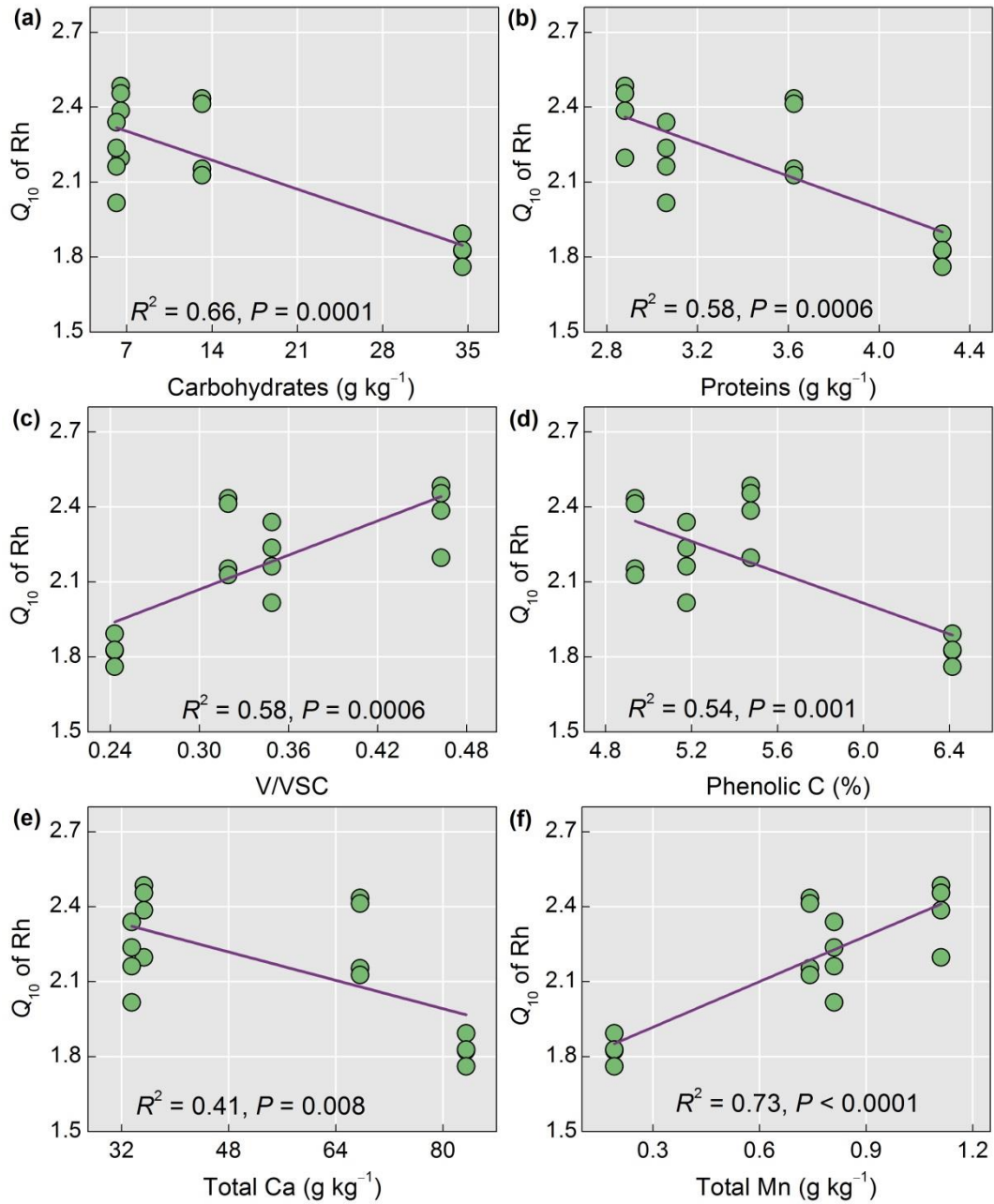


Figure 6. Relationships between the Q_{10} values of soil heterotrophic respiration (Rh) and the compost carbohydrates (a), proteins (b), ratio of vanillyl-type to total lignin (V/VSC; c), relative abundance of phenolic C (d), total calcium (Ca; e) and manganese (Mn; f) contents.

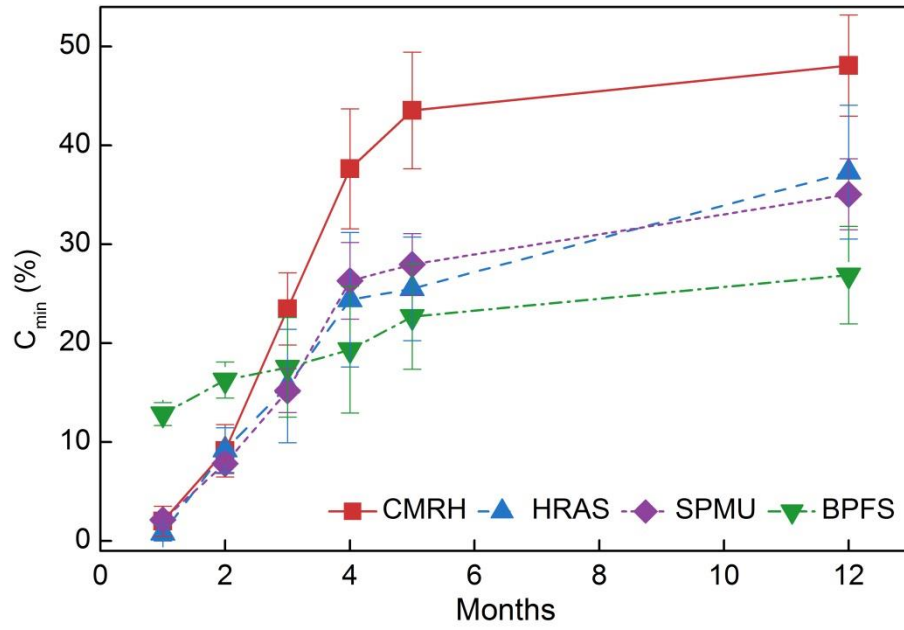


Figure 7. The percentage of compost carbon mineralized (C_{\min} , %) over different months of decomposition. Vertical bars denote standard errors ($n = 4$).