Resilience of soil functions to transient and persistent stresses is improved more by residue incorporation than the activity of earthworms

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Abstract

The development of soil sustainability is linked to the improved management of soil biota, such as earthworms, and crop residues to improve soil physical structure, enhance microbial activities, and increase nutrient cycling. This study examined the impacts of maize residue (65.8 C/N ratio, dry biomass 0.75 kg m^{-2}) incorporation and earthworms (70 g *Metaphire guillelmi* m^{-2}) on the resistance and resilience of soil C and N cycling to experimentally applied stresses. Field treatments were maize residue incorporation, maize residue incorporation with earthworm addition, and an unamended control. Resistance and resilience of C mineralization, ammonia oxidation, and potential denitrification were investigated over 28 days following a persistent stress of Cu (1 mg Cu soil g^{-1}) or a transient heat stress (50 ℃ for 16 hours). The results indicated that C mineralization was more resistant and resilient than ammonia oxidation and denitrification to either a persistent Cu or a transient heat stress. The application of maize residues significantly increased soil microbial biomass, C mineralization, ammonia oxidation and potential denitrification compared with the unamended control. Maize residues significantly improved the resistance and resilience of N processes to Cu and heat stress. The presence of earthworms significantly increased potential denitrification but had limited positive effect on functional resistance and resilience. This study suggested crop residue incorporation would strongly increase soil functional resistance and resilience to persistent and transient stresses, and thus could be a useful agricultural practice to improve soil ecosystem sustainability.

Keywords: crop residue, soil fauna, C mineralization, ammonia oxidation, denitrification

1. Introduction

Increasing soil degradation has raised awareness of soil sustainability of which a central component is the capability to withstand (resistance) and recover (resilience) from environmental stresses (Griffiths and Philippot, 2013). So much so that a global resilience programme in response to land use pressures has been suggested (Smith et al., 2016). Soil microorganisms play a central role in conferring resistance and resilience, through their central role within the soil food web and sensitivity to agricultural practices (de Vries and Shade, 2013). Crop residue amendment would increase soil organic matter (SOM), the decomposition of which provides nutrients and energy to support the growth and succession of soil biota (Shade et al., 2012). Increased SOM also leads to improved soil physical properties (Diacono and Montemurro, 2010), and accelerated carbon (C) and nitrogen (N) cycling (Turmel et al., 2015). Thus, SOM may be an important resource to strengthen the resistance and resilience of soil ecosystem (Lal, 2015).

Improving the management of soil biota, such as earthworms, in agroecosystem is an integral part of sustainable management (Fonte and Six, 2010) especially as biotic interactions of the soil food web are a critical determinant of soil function, including resistance and resilience (de

Vries and Wallenstein, 2017). Of the three ecological groups of earthworms: anecics build a relatively permanent vertical burrow system and feed on organic matter collected from the soil surface, epigeics live and feed within the soil matrix creating horizontal burrows, and endogeics inhabit the surface layers of soil consuming fresh organic matter (Brussaard et al., 2012). To varying extents earthworms mix organic matter into the soil, influence soil aggregation and porosity (Fonte and Six, 2010), gas diffusion and soil water retention, and soil microbial community structure (Bernard et al., 2012). The availability and composition of substrate provided by crop residues affects earthworm diet, behaviour and growth (Brussaard et al., 2012; Zheng et al., 2018). Experiments have shown that the interaction between earthworms and plant residues affects soil functions. Thus, earthworms regulated the ratio of C- to N- degrading enzyme activities during crop residue decomposition in a laboratory experiment (Zheng et al., 2018). Aspects of the interaction between added crop residues and earthworms have also been explored in a long-term field trial of a wheat-rice cropping system in sub-tropical China (Tao et al., 2009). Results showed that the presence of earthworms further enhanced protease and alkaline phosphatase activities in soil with incorporated maize residue (Tao et al., 2009). A comparison of bacterial community structure in the same field trial (Gong et al., 2018), showed that residue incorporation had significant effects on bacterial community structure and that earthworms increased the ratio of Proteobacteria to Acidobacteria (indicative of high nutrient turnover). Earthworms also increased the connection between taxa, which is taken as an indicator of compositional resilience (Dunne et al., 2002). The interaction between plant cover and earthworms on soil resistance and resilience was explored in a short-term greenhouse experiment, which revealed that plants rather than earthworms increased resistance and resilience to soil compaction (Griffiths et al., 2008).

To further explore such interactions, we used samples from a long-term field experiment to determine whether amendments with maize residues and earthworms affected the functional resistance and resilience of soil. We quantified changes in C mineralization, ammonia oxidation and potential denitrification rates immediately after heat- (short-term transient) and Cu- (long-term persistent) induced stress and during subsequent recovery over 28 days after Griffiths et al. (2001). Because of the identified effects of earthworms and maize residues to alter microbial community composition and increase C and N cycling in the field experiment (Gong et al., 2018), we hypothesised that soil amended with maize residues and earthworms would have greater resistance and resilience than soil amended with maize residues alone.

2. Materials and methods

2.1 Study site and soil samples

A field trial at the experimental station of Nanjing Agricultural University (China, 118º47′E, and 32º03′N) was established in 2001. In each plot, there were three treatments as described by Tao et al. (2009): maize residues (*Zea mays* L.) incorporated into soil, maize residues incorporated into soil with earthworm (*Metaphire guillelmi*) addition, and a control with no additions. Each treatment had three replicate plots, arranged in a completely randomized experimental design. Earthworms were monitored after every harvesting stage annually and were added if necessary to maintain a density of 70 g earthworm m^{-2}. This earthworm is the dominant species in this area, commonly found in disturbed arable soil and its behaviour shows it to be endogeic (Gong et al., 2018). Maize residues (0.75 kg m^{-2} air-dry weight, chopped < 2 cm) containing 7.96 g N kg^{-1}, 2.85 g P kg^{-1}, 10.67 g K kg^{-1}, and 65.8 C/N ratio were applied to the appropriate plots at the beginning at rice and wheat growth period every year.

The soil, classified as an Orthic Acrisol, was sampled in May 2016. From each plot, three surface soil samples (0-20 cm depth) approximately 10 kg in weight were randomly sampled and mixed thoroughly. The soil had a pH (H_2O) of 8.25 and contained 5.86 g C kg^{-1} and 0.71 g N kg^{-1} soil (Shu, 2018). Soil microbial biomass carbon (MBC) was analysed by chloroform fumigation (Vance et al., 1987). Mineral N (NO_3^- and NH_4^+) was extracted by shaking with 2 M KCl for 1 hour and analysed using a continuous flow analyser (Skalar San++ 4800, Netherlands). Dissolved organic carbon (DOC) was extracted following the method of Ghani et al. (2003) and analysed using a TOC analyser (Dohrmann DC-80, UK).

2.2 Resistance and resilience assay

Soils from all the treatments were packed to a bulk density of 1.1 g cm^{-3} and incubated for 7 days with a water content of 60% water-filled pore space (WFPS) at 20 ℃ prior to analysis. The stresses imposed followed Griffiths et al. (2001) and were: Cu (1 mg Cu soil g^{-1}) to provide a persistent stress; and heat (50 ℃ for 16 hours) to provide a transient stress. For the heat stressed soil, a preliminary test (Supplementary material 1) indicated that the temperature of 40 ℃ that has been typically applied in studies on temperate soils (Griffiths et al., 2001) was not a sufficient stress for these subtropical soils because of their great adaptation to a relatively high temperature (Table S1), as also found by Zhang et al. (2010). For each soil, aliquots were exposed to either a stress (heat or Cu) or were unstressed as a control, with six replicates for each field treatment and laboratory applied stress. Each aliquot contained 220 g dry-weight equivalent of soil (bulk density 1.1 g cm^{-3}) in a 500 ml capacity polypropylene pot. Six replicate aliquots of each stressed- soil were prepared by adding 2.2 ml of 1.57 M $CuSO_4 \cdot 5H_2O$ to obtain a concentration of 1 mg Cu soil g^{-1}; or 2.2 ml of sterile distilled water to both the heat-stressed and unstressed (control) soils. All the aliquots were then sealed with parafilm to exchange air but prevent any water loss. The heat- stressed soils were then incubated at 50 ℃ for 16 hours, while both Cu-stressed and unstressed soil were incubated at 20 ℃ for 16 hours. All aliquots were then incubated at 20 ℃ for the remainder of the resilience assay.

To facilitate temporal description, day 0 was defined as the time when Cu or heat was applied. Subsamples were taken for the analysis of microbial functions at intervals of 1, 3, 7, 14 and 28 days after the stresses were imposed. C mineralization was measured by the emission of CO_2 after 24 hours following the addition of 120 µl of organic C compounds which provides 50 mg C ml^{-1} and 9.72 mg N ml^{-1} to a 2 g of soil (Shu, 2018). Ammonia oxidation was determined by the chlorate inhibition method (Groffman 1985). Potential denitrification was determined following anaerobic incubation of 20 g soil in the presence of 10% (v/v) acetylene (Shu, 2018).

2.3 Data analysis

A linear mixed effect model was fit in the "lme4" package for the "R" statistical programme (version 3.5.2) using the "lmer" function (Bates et al., 2019). Effects of fixed term and random term on C mineralization, ammonia oxidation and potential denitrification were analysed. Fixed terms were field treatment, stress, time, and their interaction (treatment × stress × time). The replicate plot was considered a random term.

Stability *f(t)* was calculated as the change in biological functions of the stressed soil (*β*) compared with the unstressed control (*α*) at day *t* (Equation 1) (Zhang et al., 2010):

$$f(t) = \frac{\beta_t}{\alpha_t} \times 100 \tag{1}$$

Resistance was defined as the stability measured at day 1 after perturbation (Equation 2), while resilience was estimated as the integrative stability after day 1 up to 28 days following stress (Equation 3) (Shu, 2018).

$$Resistance = \frac{\beta_1}{\alpha_1} \times 100 \tag{2}$$

$$Resilience = \int_1^{28} f(t)dt/(28-1) \tag{3}$$

3. Results and discussion

Maize residue incorporation significantly increased the concentrations of dissolved organic C and microbial biomass C (Table 1). The linear mixed effect model demonstrated that maize residue incorporation significantly (P < 0.001) increased C mineralization, ammonia oxidation and denitrification (Table S3). These results are consistent with previous studies that maize residue addition increased microbial biomass C (Tao et al., 2009) and promoted C sequestration (Shu et al., 2015). An increased supply of nutrients, such as from the maize residues, can hasten microbial growth (Henderson et al., 2010), sustain microorganisms and enhance microbial activities (Shade et al., 2012).

When soil was stressed by either Cu or heat, C mineralization in all the treatments was more resistant and resilient than ammonia oxidation and potential denitrification (Table 2). This is consistent with several studies which demonstrated that N processes are more susceptible to external stresses than C processes (Bissett et al., 2013; Morillas et al., 2015). This may be because the microbial community carrying out C mineralization is more diverse and more functionally redundant than specialized microbial populations performing ammonia oxidation and denitrification (Philippot et al., 2013). Denitrification was particularly susceptible to the applied stresses, with resistance and resilience often less than 20% (Table 2), as also previously was shown for both Cu (Magalhães et al., 2007) and heat (Wertz et al., 2007).

We found that maize residue incorporation significantly (P < 0.05) increased the resistance and resilience of ammonia oxidation and potential denitrification to Cu (Table 2). This could be attributed to the enhanced microbial biomass (Table 1), as well as the buffering effects by adsorption or chelation of Cu^{2+} by organic matter which diminishes the bioavailability and toxicity of Cu to microorganisms (Degryse et al., 2009). The bioavailability of Cu in soil with incorporated crop residue was likely to be significantly less than in the unamended soil one day after Cu addition (Navel et al., 2010). Crop residues serve as an energy and nutrient source for microorganisms to accelerate microbial community succession and so increase microbial biomass (Brandt et al., 2010). Access to a favourable resource is important for the degree of recovery and the time that microorganisms take to recover (Placella et al., 2012). That organic amendments improved soil functional resistance and resilience to Cu has been reported previously for C mineralization in temperate soils (Gregory et al., 2009). In contrast, we observed that crop residue incorporation in this soil decreased resistance and resilience of C mineralization to Cu (Table 2). We saw that C mineralization in soil with incorporated crop residue decreased significantly after 3 days incubation in both the unstressed and the Cu stressed soil (Table S2). This could be related to the depletion of available nutrients. The different impacts of residue on the resistance and resilience of C and N processes could also be ascribed to the different stress-sensitivity and distinct microbial characteristics between C and N processes.

All the measured microbial functions were resilient to heat, especially ammonia oxidation and potential denitrification in the soil with incorporated maize residue (Table 2). Heat leads to the death of heat-sensitive microorganisms, such as proteobacteria which had a low resistance to heat stress (Frenk et al., 2017). In recovering from a transient heat stress, the attributes of the microbial communities, mixtrophy and intrinsic growth rate, determine microbial use of available C to reproduce and recolonize niches rapidly (Shade et al., 2012). Necromass, such as dead microbial cells induced by the heat stress, also provides a rapidly mineralised substrate that is easily accessible to free-living microorganisms (Drigo et al., 2012). A previous study has demonstrated that bacterial communities could recover to its original structure from a transient heat stress, but not from a persistent Cu stress (Shu, 2018). Routine successional trajectories of microbial communities may be altered differently by different stresses (de Vries and Shade, 2013), and gradual shifts of microbial community may be the result of long-term adaptations to the persistent Cu stress.

In the soils with incorporated maize residues, earthworms significantly increased potential denitrification (Table S3). Earthworms gut, casts and drilospheres are hotspots of denitrification, and thus contribute to high emission of N_2O (Lubbers et al., 2013). However, earthworm presence had few significant additional impacts on C mineralization and ammonia oxidation (Table S3). The different response between soil functions to earthworms unveils that their underlying microorganisms may be influenced by earthworms differently. For example, in a northern temperate forest in USA, earthworms enhanced cellulolytic enzyme activity and shifted soil microbial composition away from fungi and towards bacteria (Dempsey et al., 2013). Previous studies, at the site where soils were collected for this experiment, demonstrated that earthworms significantly changed the composition and connectance of the microbial community (Gong et al., 2018) and soil enzyme activities (Tao et al., 2009) when maize residues were incorporated. The lack of significant earthworm effect on resistance and resilience in this experiment, suggests that these changes were not enough to affect the stability of the soil. The effects of earthworms may also be overwhelmed by residues, further study should include a treatment of earthworms alone without residue amendment. The small effect of earthworms could result if not all the measured soils had transited through the earthworm gut, because microorganisms and their activities can be stimulated by earthworm mucus (Bernard et al., 2012). This study only focused on the bulk soil, however, Gong and colleagues (unpublished data) have found a significant effect of earthworms on the microbial community associated with soil aggregates. Therefore, it would be interesting to explore how soil resistance and resilience changes at an aggregate scale.

In conclusion, C and N processes responded differently to imposed stresses, so it is important that assays of resilience explore multiple functions and potential disturbances. Soil functions are less likely to recover from a persistent stress (e.g. Cu) than a transient stress (e.g. heat), but transient stresses can still result in a prolonged degradation to soil functions. Stresses associated with climate change, such as the frequency of long hot periods, drought or flooding could affect soil for a period after the stresses are removed. The important role of earthworms in ecosystems is widely recognised, however, in this example of a disturbed agricultural soil crop residue addition as a management option was more important than having earthworms present for restoring soil resistance and resilience. Although further research is required across a wider range of soils and with more types of residues, our findings suggest that applying crop residues to a degraded agricultural soil is a primary driver in the recovery of functions like C and N cycling that underpin productivity and sustainability.

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Table 1 Soil properties sampled at day 0 before stress imposed and the average functions in the unstressed soil over 28 days. MBC- microbial biomass carbon (μg C dry soil g^{-1}); NO_3^-- the total concentration of nitrate (μg N dry soil g^{-1}); NH_4^+- the amount of ammonia N (μg N dry soil g^{-1}); DOC- dissolved organic carbon (μg C dry soil g^{-1}). C mineralization (μg C soil g^{-1} h^{-1}), ammonia oxidation (μg N soil g^{-1} d^{-1}), potential denitrification (ng N soil g^{-1} h^{-1}) are the average in the unstressed soils during 28 days. Means±standard deviations followed by the same lowercase letters in the same column are not significantly ($P < 0.05$) different.

Treatment	MBC	NO_3^-	NH_4^+	DOC	C mineralization	Ammonia oxidation	Potential denitrification
Control	44.8±19 c	18.11±4 b	0.58±0.1 a	187.6± 6 b	17.3±0.6 b	81.5±1.9 b	255±15 b
Residue	275.3±28 a	32.43±4 a	0.67±0.01 a	338.5±10 a	31.1±1.1 a	130.5±2.5 a	1437±134 a
Residue + Earthworms	199.9±31 b	31.75±2 a	0.67±0.03 a	310.2±7 a	29.6±1.3 a	129.3±2.4 a	1432±69 a

Table 2 The resistance and resilience of C mineralization, ammonia oxidation and potential denitrification to Cu and heat. Means±standard deviations followed by the same lowercase letters in the same indicator are not significantly ($P < 0.05$) different. Field treatments were the control without any additions, maize residue incorporation, and maize residue incorporation and earthworm addition.

Treatment	C mineralization		Ammonia oxidation		Potential denitrification	
	Resistance (Cu)	Resilience (Cu)	Resistance (Cu)	Resilience (Cu)	Resistance (Cu)	Resilience (Cu)
Control	87±4 a	67±1 a	30±3 b	30±2 c	15±1 a	5±1 b
Residue	74±3 b	59±2 b	46±2 a	48±1 a	17±2 a	8±1 a
Residue + Earthworms	69±3 b	62±2 b	45±1 a	40±2 b	7±1 b	9±1 a
Treatment	Resistance (Heat)	Resilience (Heat)	Resistance (Heat)	Resilience (Heat)	Resistance (Heat)	Resilience (Heat)
Control	61±3 a	77±2 a	30±2 b	60±2 b	18±4 a	34±4 b
Residue	55±1 a	68±3 b	44±4 a	77±2 a	6±1 b	49±3 a
Residue + Earthworms	59±2 a	75±2 a	36±1 b	76±3 a	3±1 b	45±4 ab

SUPPLEMENTARY MATERIALS

Resilience of soil functions to transient and persistent stresses is improved more by residue incorporation than the activity of earthworms

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Supplementary material 1: Preliminary Experiment

Soils from the treatments of control and maize residue incorporated and earthworm added were packed to a bulk density of 1.1 g cm^{-3}, and incubated for 7 days with a water content of 60% water-filled pore space (WFPS) at 20 ℃ prior to the preliminary test. For each soil, aliquots were exposed to either a stress (heat at 40 ℃ or 50 ℃) or were unstressed as a control, with three replicates for each treatment and stress. Each aliquot contained 220 g dry-weight equivalent of soil in a 500 ml capacity pot. Three replicate aliquots of each stressed- soil were prepared by adding 2.2 ml of sterile distilled water to both the heat-stressed and unstressed control soils. All aliquots were then sealed with parafilm to exchange air but prevent any water loss. The heat- stressed soils were then incubated at either 40 or 50 ℃ for 16 hours, while the unstressed soils were incubated in a moist atmosphere at 20 ℃ for 16 hours. All aliquots were then incubated at 20 ℃ for the remainder of the same resilience assay. C mineralization, ammonia oxidation and potential denitrification were measured over 7 days following stress. The methods of C mineralization, ammonia oxidation and potential denitrification are described in main text.

Table S1 C mineralization, ammonia oxidation and potential denitrification at different field treatments and stresses. Means ± standard error of means. Field treatments were the control without any additions, and maize residue incorporation and earthworm addition.

			C mineralization (µg C soil g^{-1} h^{-1})			
Treatment	Day 1			Day 7		
	Heat 40°C	Heat 50°C	Unstressed	Heat 40°C	Heat 50°C	Unstressed
Control	16.4±0.6	11.9±1.3	20.7±1.5	12.4±0.1	9.5±0.1	12.4±0.4
Residue + Earthworms	28.7±1.4	22.2±0.1	34.9±2.6	17.7±0.4	14.2±0.1	18.8±0.2
			Ammonia oxidation (µg N soil g^{-1} d^{-1})			
Treatment	Day 1			Day 7		
	Heat 40°C	Heat 50°C	Unstressed	Heat 40°C	Heat 50°C	Unstressed
Control	88.6±4.6	33.4±0.6	95.4±2.3	93.1±1.0	51.9±2.0	104.5±1.8
Residue + Earthworms	128.1±3.0	62.1±5.2	126.0±1.3	130.3±0.3	95.4±1.3	136.3±1.1
			Potential denitrification (ng N soil g^{-1} h^{-1})			
Treatment	Day 1			Day 7		
	Heat 40°C	Heat 50°C	Unstressed	Heat 40°C	Heat 50°C	Unstressed
Control	247±10	44±14	268±83	271±42	100±85	328±16
Residue + Earthworms	959±31	28±8	1381±45	1200±44	516±171	1224±71

Table S2 C mineralization, ammonia oxidation and potential denitrification at 1, 3, 7, 14 and 28 days after Cu, heat or no stress. Field treatments were the control without any additions, maize residue incorporation, and maize residue corporation and earthworm addition. Means ± standard error of means.

	C mineralization (μg C soil g^{-1} h^{-1})			Ammonia oxidation (μg N soil g^{-1} d^{-1})			Potential denitrification (ng N soil g^{-1} h^{-1})		
Unstressed									
Day	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm
1	17.2±0.6	37.4±0.9	36.7±0.8	84.8±1.9	132.7±2.1	132.3±2.2	210.0±4.6	867.0±59.5	1652.0±46.4
3	18.2±0.4	35.9±1.1	36.4±1.5	89.0±2.9	133.1±4.1	135.9±1.9	71.0±12.0	1082.0±143.9	959.0±63.1
7	17.6±0.9	29.7±1.7	26.1±2.5	83.4±2.3	130.2±2.0	129.2±2.6	350.0±12.1	1644.0±180.2	1342.0±110.3
14	16.6±0.5	27.1±1.2	25.0±0.8	82.4±1.3	129.4±1.1	127.9±2.7	201.0±21.1	1410.0±152.1	1288.0±51.5
28	16.9±0.6	25.1±0.8	24.0±1.1	68.1±1.1	126.8±3.4	121.0±2.5	441.0±26.9	2179.0±137.8	1920.0±74.9
Cu-stressed									
Day	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm
1	14.9±0.6	27.8±1.1	25.5±1.3	25.4±2.4	61.7±3.7	59.0±1.4	32.3±1.7	141.5±11.3	118.7±14.7
3	13.1±0.4	23.9±0.6	24.7±1.8	27.9±2.0	75.3±3.5	63.6±3.9	12.4±1.2	53.5±7.5	30.7±4.5
7	11.0±0.4	16.3±0.9	16.9±1.1	26.7±4.5	63.3±2.2	58.5±5.2	11.1±3.3	103.2±19.4	115.2±10.8
14	10.6±0.4	15.1±0.5	14.2±0.8	24.1±3.2	63.0±2.2	47.6±4.2	6.1±1.2	149.1±18.9	122.6±16.7
28	12.0±0.2	15.0±0.4	16.2±0.9	20.9±2.3	55.8±3.7	44.5±0.9	10.7±1.3	142.7±44.5	181.5±44.1
Heat-stressed									
Day	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm	Control	Residue	Residue + Earthworm
1	10.3±0.3	20.6±0.5	21.7±0.5	25.0±1.4	58.8±5.0	47.2±1.3	36.8±8.0	53.7±4.6	48.4±5.4
3	11.7±0.2	18.7±0.6	18.0±0.7	49.7±2.5	106.7±4.4	106.3±4.2	20.6±2.9	114.5±35.5	89.8±17.2
7	14.1±1.2	18.4±1.0	19.9±0.3	47.0±2.0	95.8±4.4	93.3±1.8	81.5±19.6	788.6±94.2	678.3±88.3
14	13.1±0.2	18.0±0.8	19.3±0.4	50.1±2.2	102.9±2.8	99.0±4.5	70.3±13.1	864.2±123.8	689.4±85.8
28	13.9±0.4	20.8±0.9	20.1±0.7	45.4±1.3	100.6±4.7	97.7±3.1	196.6±19.8	1303.9±150.6	966.4±119.4

Table S3 Coefficients (estimate ± standard deviation) and R^2 values for the linear mixed effect model to evaluate the main and interaction effect of field treatment, stress and time on C mineralization, ammonia oxidation, and potential denitrification. Contributions of all the fixed effects and random effects to the complete model were evaluated as R^2. *, **, *** indicates the significance at $P < 0.05$, 0.01, 0.001, respectively.

	Fixed term	C mineralization (μg C soil g^{-1} h^{-1})	Ammonia oxidation (μg N soil g^{-1} d^{-1})	Potential denitrification (ng N soil g^{-1} h^{-1})
Main effect	Intercept	17.7±1.0 ***	88.8±3.8 ***	153.8±76.1 *
	Residue	18.1±1.4 ***	44.1±5.4 ***	833.1±107.6 ***
	Earthworm and Residue	16.9±1.4 ***	45.6±5.4 ***	1060.5±107.6 ***
	Cu	-4.5±1.2 ***	-61.4±4.2 ***	-134.1±76.5 *
	Heat	-6.1±1.2 ***	-49.2±4.2 ***	-136.1±76.5 *
	Day	0.0±0.1	-0.7±0.2 **	9.5±3.8
Interaction effect	Residue × Cu	-7.3±1.6 ***	-3.0±6.0	-753.8±108.3 ***
	Earthworm and Residue × Cu	-6.9±1.6 ***	-11.1±6.0	-1006.2±108.3 ***
	Residue × Heat	-10.7±1.6 ***	0.8±6.0	-701.3±108.3 ***
	Earhtworm and Residue × Heat	-8.6±1.6 ***	-6.2±6.0	-929.6±108.3 ***
	Residue × Day	-0.4±0.1 ***	0.5±0.3	32.9±5.3 ***
	Earthworm and Residue × Day	-0.4±0.1 ***	0.2±0.3	11.1±5.3 *
	Cu × Day	0.0±0.1	0.5±0.3	-10.0±5.3
	Heat × Day	0.1±0.1	1.0±0.3 ***	-3.5±5.3
	Residue × Cu × Day	0.1±0.1	-0.7±0.4	-30.6±7.5 ***
	Residue and Earthworm × Cu × Day	0.2±0.1	-0.7±0.4	-6.8±7.5
	Residue × Heat × Day	0.3±0.1 **	0.0±0.4	6.0±7.5
	Residue and Earthworm × Heat × Day	0.3±0.1 **	0.4±0.4	15.6±7.5 *
	Fixed effect R^2	0.82	0.90	0.87
	Random effect R^2	0.01	0.01	0.02

*Note: the structure of linear mixed model: function ~ Field treatment * Stress * Day + Random effect from replicates.*