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Title: Non-additive responses of soil C and N to rice straw and hairy vetch (*Vicia villosa roth L*) mixtures in a paddy soil

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Abstract:

Aims We studied the effects of mixing rice straw and hairy vetch plant residues in a subtropical paddy soil, on subsequent carbon (C) and nitrogen (N) dynamics.

Methods Using a theoretical framework, we designed two groups of experiments (involving equal amounts of residual C or N addition, referred to as either C or N treatments). Each experiment included mixed residues of rice straw and hairy vetch at different mixing ratios. Soils together with residues were incubated at 25℃ under waterlogged conditions for 100 days. Greenhouse gas (GHG) emissions and available C and N fractions were measured continuously.

Results Both C and N treatments affected soil C and N dynamics, and these dynamics were quantitatively dependent on residue C/N ratios. The effect of residue mixtures on C and N dynamics could not be predicted from single residues, since there were non-additive effects of residue mixtures. Synergistic effects were generally more frequent than antagonistic effects. Residue mixtures tended to enhance CO_2 and CH_4 emissions in both C and N treatments but decreased N_2O emissions in the N treatment. In the N treatment, dissolved organic C (DOC), dissolved organic N (DON), and microbial biomass C (MBC) concentrations increased. DOC and DON concentrations decreased in the C treatment. Residue mixtures enhanced the global warming potentials (GWP) of greenhouse gases (GHG) emitted from soil by non-additive synergistic effects. The C/N ratio of residue mixtures affected the non-additive responses of soil C and N dynamics, for example mixtures with a C/N ratio of 25 had higher CO_2 emissions and DOC concentrations than those with a C/N ratio of 35 as a consequence of non-additive effects, however, CH_4 emissions and MBC concentrations were higher in mixtures with a C/N ratio of 35 than in mixtures with a C/N ration of 25.

Conclusions These results indicated that non-additive effects can impact soil C and N dynamics and

that residue C/N ratios play an important role in influencing non-additive effects. Applying a single residue to paddy soils may be better than residue mixtures from a GHG mitigation perspective.

Key words: Residue mixtures, C/N ratio, Soil C and N dynamics, Non-additive response, Paddy soil

Introduction

Rice straw and green manure applications are important practices for managing soil fertility in the South of China. They can regulate soil carbon (C) and nitrogen (N) cycling, affect plant growth and change microbial community structure (Ma et al. 2009; Hansen et al. 2017). Previous research on rice straw and green manure applications alone have identified factors that influence the decomposition rate, such as C/N ratio (Huang et al. 2004), chemical composition (Redin et al. 2014), amount of input (Kimura et al. 2004), and soil properties (Wang et al. 2013). Nowadays, it is normal practice to return rice straw and green manures together to paddy fields. Consequently, it is valuable and necessary to study the mixtures of rice and green manure residues in order to understand the factors affecting decomposition processes.

Previous research has shown that residue mixtures may have additive or non-additive effects on decomposition processes, and that non-additive effects predominate (Hättenschwiler et al. 2005; Chen et al. 2015, 2017). An additive effect implies that there is no interaction between the constituent residues during decomposition. Accordingly, the decomposition processes of the residue mixture, e.g. decomposition rate, can be predicted from the decomposition rates of individual components of residue mixture. A non-additive effect occurs when there are interactions between the component residues, which either stimulate (i.e. synergistic) or inhibit (i.e. antagonistic) the decomposition processes in the residue mixture leading to differences from predictions based on the decomposition of the constituent residues (Chen et al. 2017). It can be unreliable to predict the decomposition of residue mixtures from knowledge of the decomposition of single residue components.

Although Hättenschwiler et al. (2005) have reviewed three plausible mechanisms for non-additive effects on C and N dynamics (i.e. the nutrient transfer theory, the effects of specific compounds theory and the improved micro-environmental conditions and trophic levels theory), the mechanisms of this process remain unclear (Gartner and Cardon, 2004; Makkonen et al. 2013; Chen et al. 2017). Tardif and Shipley (2015) suggested that all mechanisms of non-additive effects stem from the chemical or structural differences of residues in the mixture rather than their taxonomic identity. Various chemical components released from residue mixtures can impact on microbial growth and activity in different ways, ultimately affecting the decomposition process positively or negatively (Sinsabaugh et al. 2002). Recent studies on the decomposition of residue mixtures have indicated that variations in chemical characteristics can influence the effects of residue-mixing on soil C and N cycling (Lecerf et al. 2011; Chen et al. 2017). However, very few studies have focused on how specific chemical characteristics (such as the C/N ratio) of the residue mixture can affect C and N dynamics in the decomposition process, which is important for revealing the mechanism of residue-mixing effects (Bonanomi et al. 2010).

The primary objective of this study was to assess the effects of incorporation of rice straw and green manure (hairy vetch) mixtures on soil C and N dynamics, and to evaluate residue mixing-effects as influenced by the C/N ratio of residues in the mixture. Laboratory incubation experiments (applying equal amounts of residual C and N, respectively) with rice straw, hairy vetch and their mixtures were conducted to answer a set of research questions: (1) does the type or composition of crop residues affect soil C and N dynamics? (2) if yes, does the C/N ratio correlate with decomposition processes? (3)

do non-additive effects (synergistic and antagonistic effects) on soil C and N dynamics occur? and, (4) does the C/N ratio influence non-additive responses of soil C and N dynamics in residue mixtures?

Material and methods

Soil and residues

Soil was collected from an experimental site managed by the National Engineering and Technology Research Center for Red Soil Improvement in Fengcheng, Jiangxi Province, China (N28◦07′, E115◦56′ and altitude 25.4 m). The soil is derived from quaternary parent materials, with a pH of 5.2 and a texture of 70.1% sand, 27.1% silt, and 2.8% clay in the upper layer (0-20 cm). Other soil properties were as follows: soil organic C (SOC) 24.3 g kg^{-1}, total N (TN) 2.3 g kg^{-1}, mineral N (N_{min}) 71.5 mg kg^{-1}. In early April 2015, soil at depths of 0～20 cm was collected from ten locations in a paddy field along an "S" shaped transect. All soil samples were mixed thoroughly, air dried, crushed, passed through a 2-mm sieve, cleared of visible roots and stones, and stored in sealed containers before pre-incubation.

At the same location as the soil, aboveground biomass of the rice straw (*Oryza sativa L.*, hereafter abbreviated as RS) and green manure (hairy vetch, *Vicia villosa roth L.*, hereafter abbreviated as GM) residues were collected from ten points, then dried in a fan oven at 60 °C for 24 h, ground and sieved to particle size < 1mm, and stored in sealed containers.

Incubation experiment

The air-dried paddy soil was rewetted to 60% water holding capacity and pre-incubated at 25 °C for 10 days in dark so as to activate the soil microbes (Wang et al. 2013). After pre-incubation, the soil (200.0 g equivalent dry-weight) was placed in a plastic basin and amended with residues. It was then hand-mixed thoroughly and placed in 500 ml culture-flasks.

There were two treatment groups in this experiments; the C and N groups, and each group included five treatments: control (no residues) (CK); hairy vetch alone (C1 or N1); hairy vetch and rice straw added together with a residual C/N ratio of 25 (C2 or N2); hairy vetch and rice straw added together with a residual C/N ratio of 35 (C3 or N3); and rice straw alone (C4 or N4). The amounts of C and N in the residues added to soil were 164.5 mg pot^{-1} and 11.9 mg pot^{-1} for the C treatments and the N treatments, respectively. A C/N ratio of 25 has been identified as optimal for microbial decomposition of residues (Parnas, 1976; Ndegwa and Thompson, 2000). The C/N ratio of 35 was almost equal to that of mixture of the rice straw and hairy vetch applied in the fields. The amount of hairy vetch added to the soil was double that normally returned to fields. Thirty-three replicates of each treatment were prepared. Detailed information describing the different mixtures is provided in Table 1. Distilled water was added to each flask to maintain a 2-cm depth of water above the soil surface (1:1 water/dried soil w/w). All flasks were sealed by a rubber septum and incubated at 25 °C in a growth chamber in the dark. During the experiment, each flask was opened to allow gas exchange for 30 min after sampling. The water depth was kept constant by adding water to flasks every 5 days. Three randomly selected replicates of each treatment were destructively sampled at 1, 3, 5, 10, 15, 20, 30, 45, 60, 75 and 100 days after incubation for analysis of soil chemical and microbial properties.

The emissions of CO_2, CH_4, and N_2O were measured at 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 days after the start of the incubation. To do this, a 20 ml gas sample was collected from each flask with a plastic syringe and was then was injected into an evacuated 12 ml glass vial fitted with rubber stoppers to subsequently measure CO_2, CH_4, and N_2O

concentrations. All gas samples were analyzed within 24 h after sampling.

Residues and soil C and N characteristics analyses

The total C and N content of crop residues and physicochemical properties of the soil were determined according to the Chinese Soil Society guidelines (Lu, 2000). Dissolved organic C (DOC), total dissolved N (TDN), microbial biomass C (MBC), microbial biomass N (MBN) and mineral N (N_{min}: NH_4^+-N and NO_3^--N) in the incubated soil were measured. Sixty g fresh-weight of soil was extracted with 120 ml double distilled water (Wang et al. 2013), the extraction was passed through 0.45-μm filter paper and analyzed using a TOC/N analyzer (Aanalytikjena, Multi N/C 2100, Germany) and an autoanalyzer (SEAL AutoAnalyzer3, Germany). Dissolved organic N (DON) was calculated as the difference between TDN and the inorganic N. MBC and MBN were estimated by the chloroform-fumigation extraction method (Brookes et al. 1985; Davidson et al. 1989). N_{min} was determined using an autoanalyzer.

Concentrations of CO_2, CH_4 and N_2O in the gas samples were measured using a gas chromatograph equipped with flame ionization (FID) and electron capture detectors (ECD) (Agilent 7890A, USA).

Data analysis and statistics

Emissions of CO_2, CH_4, or N_2O were calculated using the formula E1:

$$F(X) = \frac{(A-B)\times V\times M\times 273\times 1000}{22.4\times m\times t\times (273+T)} \qquad \text{(E1)}$$

where *F(X)* is the emission flux of *X* gas (mg *X* kg^{-1} d^{-1}); where A and B stands for *X* concentration (*X* Air^{-1}, mol×10^{-6} mol^{-1}) in the samples collected at the beginning and at the end, respectively; *V* is the volume of gas in pot (L); *M* is the molar mass of *X* (g mol^{-1}); *t* is the number of days in its sampling interval; *m* is the weight of dry-soil and *T* is mean temperature (°C) in pot, 273 is absolute temperature (K) and 22.4 is the molar volume of gas under standardized state (L mol^{-1}).

Cumulative emissions of CO_2, CH_4, or N_2O was computed using formula E2 (Chen et al. 2015):

$$C_{t'} = C_t + \frac{F_t + F_{t'}}{2} \times (t' - t) \qquad \text{(E2)}$$

where $C_{t'}$ and C_t are gas accumulation (mg kg^{-1}) at t' and t, respectively; $F_{t'}$ and F_t are the emissions (mg kg^{-1} d^{-1}) at t' and t; t and t' are the sampling time and the next sampling time after t (d).

The Global warming potentials (GWPs, mg CO_2 equivalents kg^{-1}) of different treatments were calculated using formula E3:

$$GWP = CO_2 + CH_4 \times 25 + N_2O \times 298 \qquad \text{(E3)}$$

based on a 100-year time frame, the GWP coefficients of CH_4 and N_2O are 25 and 298, respectively, when the GWP value of CO_2 is assumed to be 1 (Forster et al. 2007).

To determine whether the residue-mixing effects (*RME*) on soil C and N occurred, the following equation (E4) was used (Hoorens et al. 2003):

$$RME = \left(\frac{OBS\ \text{value}}{EXP\ \text{value}}\right) - 1 \qquad \text{(E4)}$$

where *OBS* is the measured value of a soil C or N transformation (e.g., soil CO_2/CH_4/N_2O emission, dynamics of DOC, DON etc.), and *EXP* was calculated by averaging the results of the respective single residue treatments according to the following equation E5 (Meier and Bowman, 2010):

$$EXP\ \text{value} = \sum_{i+1}^{S} R_i / S \qquad \text{(E5)}$$

where *Ri* is the soil response when residue *i* was added alone, and *S* is the total number of types in the

residue mixtures. Significant differences between *RME* and zero indicate that non-additive effects occur. The strongest synergistic effects would lead to the greatest positive departure from zero and the strongest antagonistic effects would lead to the greatest negative departure from zero.

Analogous to the calculation of the N_2O emission factor in IPCC (2000), we defined the CO_2-C or CH_4-C emissions per unit C amendment and N_2O–N emissions per unit N amendment as an emission fraction (EF) and calculated the EF for residue amendments using equations E6 and E7 (Huang et al. 2004):

$$EF_{CO2\ or\ CH4} = [(\Sigma CO_2\ or\ CH_4\_T - \Sigma CO_2\ or\ CH_4\_C)/T_C] \times 100\% \quad \text{(E6)}$$

$$EF_{N2O} = [(\Sigma N_2O\ \_T - \Sigma N_2O\ \_C)/T_N] \times 100\% \quad \text{(E7)}$$

where $\Sigma CO_2\ or\ CH_4\_T$ *and* $\Sigma N_2O\ \_T$ are cumulative CO_2-C or CH_4-C and N_2O-N emitted from residue-treated soils, respectively; $\Sigma CO_2\ or\ CH_4\_C$ and $\Sigma N_2O\ \_C$ are cumulative CO_2-C or CH_4-C and N_2O-N emitted from control (no residue), respectively; T_C and T_N are the content of residue C and N, respectively.

The differences in soil CO_2, CH_4, N_2O fluxes, DOC, DON, MBC, MBN, N_{min} concentrations among different treatments and groups (equal C and equal N) were tested by a two-way ANOVA. One-way ANOVA followed by Duncan's multiple comparisons were used for evaluating the statistical differences in soil C and N between treatments. This analysis was used to test the differences in soil CO_2, CH_4, N_2O fluxes, DOC, DON, MBC, MBN, N_{min} concentrations between different treatments and incubation times. A paired t-test was used to assess significant differences in CO_2, CH_4 and N_2O concentrations between treated and untreated flasks (no soil), and whether the residue-mixing effect differed significantly from zero (Bonanomi et al. 2010). Regression analysis was conducted to examine the relationships between residue C/N ratios and CO_2, CH_4 and N_2O emissions with DOC, DON, MBC, MBN, N_{min} concentrations (Huang et al. 2004). All statistical analyses were conducted using SAS 8.0 with a significance level of $P < 0.05$.

Results

Dynamics of greenhouse gas (GHG) fluxes and soil C and N concentrations during residues decomposition

The incorporation of crop residues had an immediate effect on the emissions of CO_2, and N_2O (Fig. 1). The CO_2 fluxes from all residue-treated soils showed similar patterns, with emissions increasing to a peak after about one week and then decreasing steadily during the incubation period. However, in the N treatments, e.g. N2, N3 and N4, there was a secondary peak after about the 40^{th} day. Residue amendment also increased CH_4 fluxes. Net emission of CH_4 wasn't detected in the initial stage, but reached a peak in the N treatments (especially in N3 and N4). The emissions of CH_4 were distinctly different in the C and N treatments over the first 40 days, with a general rise in emissions in the C treatments, but in the N treatments following and initial increase in emissions, there was a sharp decline at around day 40. In contrast to CO_2 and CH_4 fluxes residue amendment decreased N_2O fluxes, especially after 60 days. The N_2O fluxes from residue-treated soils in the 2 treatments showed a similar pattern, with an initial decrease followed by more stable emissions during the period between day 20 to 40, and then increasing to a plateau beyond day 40.

Soil C and N concentrations in control and residue-treated soils are shown in Fig. 2. In general, residues amendment increased DOC, DON, MBC and MBN concentrations, where the C/N ratio was $\leq$35 (including C1, C2, C3, N1, N2 and N3) but where the C/N was 63 (C4 and N4) N_{min} concentrations decreased, compared with the control. Supplementary information (Table S1) describes

the average concentrations of C and N on all sampling dates, and an ANOVA analysis shows that there were significant differences in DOC, DON and N_{min} among residue C/N ratio. The two-way ANOVA also showed that there were significant differences in the GHG fluxes and soil chemistries between litter treatments and incubation times (Table 2).

The effects of residue C/N ratio on GHG emission fractions and soil C and N concentrations

A two-way ANOVA showed that there were significant differences in GHG emissions between residues and C/N ratios (Table S1). Here, we used the gas emission fractions (EF) rather than the cumulative gas emissions since was able to better reflect GHG emission potentials during residues decomposition (IPCC, 2000). Values of the EF_C (EF in the C treatment) and EF_N (EF in the N treatment) indicated that each gas EF in the C and N treatments was not a constant, but dependent on the residue's C/N ratio (Fig. 3). The CO_2 EF in the N treatment was negatively related to residue C/N ratio, but not correlated in the C treatment. Interestingly, in the two treatments, the quadratic curve fitted well with the relationship between the CH_4 EF and residue C/N ratio, indicating that there was an optimal C/N ratio which could cause the largest CH_4 EF. The N_2O EF in the C and N treatments were always negatively related to residue C/N ratio.

At the end of incubation, simple fitting curve could intuitively describe the tendency of soil C and N concentrations and the residue C/N ratio to affect GHG emissions (Fig. 4). In particular, although the MBC concentration could be modelled well by a quadratic function, the DOC, DON, MBN and N_{min} concentrations in C treatments were negatively correlated with residue C/N ratio. However, in N treatments, the DOC and DON concentrations fitted a quadratic curve well, and the MBC concentration was positively correlated with the residue C/N ratio but MBN and N_{min} were negatively associated to residue C/N ratio.

Residue-mixing effects on greenhouse gas emissions and soil C and N concentrations

The bivariate relationship between observed and expected values showed that non-additive effects were more frequent than additive effects throughout the incubation period (Fig. 5, 88 cases for CO_2 and N_2O in total; 68 cases for CH_4 in total; 44 cases for DOC, DON, MBC, MBN and N_{min} in total). For CO_2, CH_4, N_2O, DOC, DON and MBC, 51.1%, 85.3%, 71.6%, 70.5%, 63.6% and 61.4% of cases showed non-additive effects, and 53.3%, 53.4%, 50.8%, 51.2%, 51.6% and 66.7% were synergistic respectively; while for MBN and N_{min}, 72.7% and 54.5% of cases showed additive effects, respectively.

Throughout the incubation period, the average strength of residue-mixing effects for CO_2, CH_4, N_2O, DOC, DON, MBC, MBN and N_{min} in the residue mixture treatments are presented in Fig. 6. The results showed that residue mixtures significantly increased CO_2 and CH_4 fluxes by 3.6% and 14.2% in the C treatment, 6.1% and 13.3% in the N treatments, and decreased N_2O fluxes by 3.9% in the N treatment. Interestingly, residue mixtures decreased DOC and DON in the C trial but increased them in the N treatment.

Relationship of residue mixtures C/N ratio and residue-mixing effects

The relationships between the residue mixture C/N ratios and the strength of residue-mixing effects was demonstrated by non-additive effects on cumulative GHG emissions, global warming potentials (GWP) and the final soil C and N concentrations (Fig. 7). For example, non-additive synergistic effects were observed in three of the four residue mixtures for soil CO_2 (C2, N2 and N3), CH_4 (C3, N2 and N3) and N_2O (C1, C2 and C3) emissions, were found in all residue mixtures for GWP, and for soil DOC, DON and MBC concentrations in all of the N treatments; two synergistic effects and

two antagonistic effects were found for soil N_{min}. In general, synergistic effects were more frequent than antagonistic effects within residue mixtures on soil C and N dynamics (Fig. 7).

A two-way ANOVA (Table 3) also showed that there were significant differences in residue-mixing effects between residue C/N ratios and treatments. Combined with the observations in Fig. 7, marked differences in residue-mixing effects on soil C and N fluxes between the C and N treatments were found, indicating that more non-additive synergistic effects occurred in the N treatment. Significant differences in residue-mixing effects were apparent between C/N ratios of 25 and 35, showing that non-additive effects tended to be synergistic for CO_2 and N_2O at a C/N ratio of 25, and for CH_4 and MBC at C/N 35. However, the C/N ratio of residue mixtures had slight residue-mixing effects on GWP (Table 3).

Discussion

Effects of residues amendment on soil C and N dynamics

In this study, marked effects on soil C and N dynamics were found in soil treated with single or mixed residues (Figs. 1, 2 and Table S1). It could be seen from the control that the paddy soil was a "source" of CO_2, CH_4 and N_2O (Fig. 1a, b, c and Table S1), and that residue application dramatically enhanced CO_2 and CH_4 but inhibited N_2O emissions. Similar results have been reported in other paddy soils (Ma et al. 2009; Liu et al. 2014; Ye and Horwath, 2017). The enhanced emissions may be due to the increased soil microbial biomass and the growth of particular methanogenic populations after residues incorporation, which often stimulated CO_2 and CH_4 emissions (Lou et al. 2004; Conrad and Klose, 2006). In addition, anaerobic decomposition of residues does not only supply methanogenic substrates but also reduces the soil oxidation-reduction potential (Eh) which may favor CH_4 production (Cai et al. 1997; Ma et al. 2009). Kludze et al. (1993) found that soil would not emit CH_4 until its Eh was less than -150 mV, which might be the reason why there was no net CH_4 emission at the initial stage of incubation. The decreased N_2O emission might be ascribed to the development of a more anaerobic environment in the presence of residues (Cai et al. 1997; Ma et al. 2009), which decreased the substrate (nitrate nitrogen) for denitrification and favored full reduction of N_2O to N_2.

Residues decomposition can form dissolved organic matter (DOM) in natural and farmland ecosystems (Kalbitz et al. 2000; Zhu et al. 2014). However, some studies have claimed that residue application did not dramatically enhance soil DOM concentrations, because of the vulnerability of DOM released from residues which could be decomposed and utilized in the short time (Jiang et al. 2013). Hagedorn et al. (2004) reported that about 70% of DOM was extracted from soil old organic matter pools, and any methods that activate the soil C and N pool could increase soil DOM concentrations. In our study, the application of single or mixed residue also increased soil DOC, DON and MBC concentrations compared to the control (Fig. 2 and Table S1). Thus, it is reasonable to conclude that residue application could activate the soil organic C or N pool and increase the soil microbial community in a subtropical paddy soil.

Soil N_{min} concentrations were significantly higher in C1, C2, C3, N1, N2 and N3 treatments but lower in C4 and N4 treatments than that in the control (Fig. 2 and Table S1). When soil N is deficient and limits microbial growth, the residue N content would play an important role in controlling the decomposition process, and determining the balance between N mineralization and immobilization (Recous et al. 1995; Jensen et al. 2005). The enhanced N_{min} concentrations in the C1, C2 and C3 treatments may have been due to the relatively higher initial residue N content compared to C4, leading to a higher availability of N for soil microbial decomposers and resulting in more inorganic N

297 production. However, although there were equal amounts of residue N in each treatment of N group,
298 significant differences of soil N_{min} concentrations were observed. This result could be interpreted by the
299 coupling C and N cycles in which the C content of residues determined the balance between
300 mineralization and immobilization (Soussana and Lemaire, 2014).

301 **Responses of soil C and N dynamics to residue C/N ratio and mixing effects**

302 Residue C/N ratios influenced soil C and N dynamics (Huang et al. 2004). Particularly, for the
303 CO_2 emission fraction (EF), different relationships between residue C/N ratio and the CO_2 EF were
304 found in the two experiments (Fig. 3), indicating that residues C or N content could change the effects
305 of C/N ratio on CO_2 emission. Huang et al. (2004) reported that residues producing more DOM could
306 result in higher CO_2 emissions, because of the vulnerability of DOM to bio-mineralization. Our study
307 also found a positive and significant relationship between DOM (including DOC and DON) and CO_2
308 emissions (Table S2). Residues with a lower C/N ratio or with a higher C content would produce more
309 DOM (Heal et al. 1997; Mungai and Motavalli, 2006), leading to an enhancement of CO_2 emissions.
310 However, in all treatments the relationship between residue C/N ratio and the two GHG (CH_4 and N_2O)
311 EFs showed similar responses (Fig. 3), indicating that residue C/N ratios might control production.
312 This is partly supported by Ding et al. (2004) and Huang et al. (2004) who reported that soil CH_4 and
313 N_2O emissions were altered by soil DOM and N_{min}, and Table S2 also confirmed this relationship.
314 Application of residues with a lower C/N ratio or with a higher C content, leading to more DOM,
315 would result in more anoxic conditions which are favorable for methanogenesis (Baggs and Blum,
316 2004). Moreover, Krüger and Frenzel (2003) reported that any agricultural treatments enhancing the N
317 level would increase the community of CH_4 oxidizing bacteria and hence decrease CH_4 emissions.
318 These results indicate that higher DOM and lower N_{min} concentrations would result in the highest CH_4
319 emission, which could explain the observed relationships between C/N ratio and CH_4 EF (Fig. 3). Heal
320 et al. (1997) explained that residues with a lower C/N ratio decomposed more rapidly and released
321 more N_{min}, and consequently produced more substrate for N_2O production by denitrification (Huang et
322 al. 2004).

323 For soil C and N concentrations, our observations are generally consistent with previous studies
324 suggesting DOC, DON, MBN and N_{min} are negatively correlated to residue C/N ratio under equal
325 amounts of residue or C, because lower C/N ratio residues always have more N, and are more easily
326 decomposed (Huang et al. 2004; Rousk and Bååth, 2007; Marschner et al. 2015; Ye and Horwath,
327 2017). However, the C/N ratio-dependent MBC content curve showed that there was an optimum C/N
328 ratio for the microbial biomass. This may be partly explained by the non-additive synergistic effects of
329 mixtures (Abouelenien et al. 2014; Chen et al. 2017). Furthermore, the processes of change rules of
330 DOC, DON and MBC were strongly influenced by residue C/N ratio in N treatments but these were
331 different from the C treatments. This difference may arise from: a) the different C and N contents: the
332 effect of residues with higher C content can be greater than that with a lower C/N ratio and lower C
333 content; and b) the different non-additive effects: there were more non-additive synergistic effects in
334 the N treatments than that in the C treatments (Fig. 7).

335 Many studies have shown that effects of mixed residues can not be summarized from the
336 component species because of the existence of interactive effects (Gartner and Cardon 2004;
337 Hättenschwiler et al. 2005; Chen et al. 2017). Our results confirmed the predominance of non-additive
338 effects that arose from residue mixtures influencing soil C and N dynamics (Fig. 5). Overall, for CO_2,
339 CH_4, DOC, DON and MBC release or turnover (Fig. 5a, b, d, e and f), synergistic effects were far more
340 frequent than antagonistic effects, which indicated that residue mixtures were more likely to increase

these five processes by non-additive effects. Abouelenien et al. (2014) reported that residue mixtures usually had a more balanced nutrient composition, which would provide a more suitable habitat for microorganisms, leading to a higher soil respiration and mineralization. However, Fig. 6 showed different non-additive effects on DOC and DON in the two main treatments. Residue mixtures probably inhibited the two processes in the C treatment while they were promoted in the N treatment, with an indication that the quantity of C and N in residue mixtures influences the non-additive effects.

Previous information showed that synergistic effects on nutrient release from residue mixtures were predominant (Gartner and Cardon 2004; Lecerf et al. 2011). These observations imply that residue mixtures are more beneficial to improve soil microbial activity than a single residues (Nayono et al. 2010; Abouelenien et al. 2014), probably resulting in enhanced C mineralization and a lower Eh, which wuold favor CH_4 production but inhibit N_2O emissions (Lou et al. 2004; Ma et al. 2009), which is consistent with our results. However, our results also provided some contrasts with recent studies. For instance, Chen et al. (2017) reported that residue-mixing effects on MBN and N_{min} were non-additives and reported antagonistic effects on the MBC, whereas our work showed the reverse. These differences may result from: (a) different species of residues used in the incubation; and (b) different quantities of C or N in residues used in incubation.

In present study, the strength of residue-mixing effects on CO_2, CH_4, DOC, DON and MBC were controlled to a large extent by the residue mixture's C/N ratio (Table 3, Fig.7). Residue mixtures with a C/N ratio of 25 would have higher CO_2 emissions and DOC content than those with a C/N ratio of 35, but lower CH_4 emissions and MBC contents (Fig. 7). The possible reasons may be: (a) compared to residue mixtures with a C/N ratio of 35, microbial populations in mixtures with a C/N ratio of 25 are more likely to have increased access to N pools which in turn will enable soil C mineralization by non-additive processes; (b) the chemical heterogeneity of residue mixtures with C/N ratios of 25 and 35 may be different, causing different non-additive effects on soil C and N processes (Harguindeguy et al. 2008); and (c) residue mixtures with a C/N ratio of 35 could increase synergistic effects by creating the optimum conditions for the hydrolysis-acidogenic phase of microorganism growth and reproduction (Nurliyana et al. 2015). Residues mixtures generally increased the net GHG emissions from soils as a result of impacts on CO_2, CH_4 and N_2O emissions. The C3 and N3 treatments were associated with the lowest overall GWP mostly as a consequence of the low rates of CO_2 or N_2O emissions occurring and the high residue C/N ratio. However, future research should explore these explanations by designing experiments with more C/N ratios to establish wider relationships between residue mixtures and soil C and N dynamics.

Conclusions

Equal amounts of residue C or N application increased paddy soil CO_2 and CH_4 emissions, GWP and DOC, DON and MBC concentrations, whilst inhibiting N_2O emissions. Most of these changes, including MBN and N_{min}, were quantitatively dependent on residue C/N ratio or their absolute C and N contents. Additionally, non-additive (synergistic and antagonistic) effects of residue mixtures on soil C and N dynamics occurred frequently; in particular, synergistic effects were more frequent than antagonistic effects. Residue mixtures generally enhanced the GWP of greenhouse gases emitted from soil by non-additive synergistic effects. Therefore, non-additive effects impact soil C and N dynamics and residue C/N ratio may play an important role in influencing non-additive effects through mechanisms such as priming on soil C and N dynamics. Application of a single residue to paddy soils may be better than residue mixtures from a GHG mitigation perspective.

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Table 2 Two-way ANOVA for CO_2, CH_4 and N_2O fluxes, DOC, DON, MBC, MBN and N_{min} between different treatments and incubation times. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N_{min}, mineral nitrogen.

Sources	SS	df	*F*	*P*
%CO_2				
Treatments	49699.20	7	252.13	<0.0001
Incubation time	12392.11	21	337.06	<0.0001
Treatments × Incubation time	25307.47	147	24.52	<0.0001
%CH_4[a]				
Treatments	418.48	7	2339.98	<0.0001
Incubation time	69.60	16	170.26	<0.0001
Treatments × Incubation time	279.24	112	97.59	<0.0001
%N_2O				
Treatments	4.72	7	155.00	<0.0001
Incubation time	98.01	21	1072.26	<0.0001
Treatments × Incubation time	19.56	147	30.57	<0.0001
%DOC				
Treatments	139107.96	7	228.19	<0.0001
Incubation time	131851.08	10	151.40	<0.0001
Treatments × Incubation time	111398.07	70	18.27	<0.0001
%DON				
Treatments	17599.49	7	375.08	<0.0001
Incubation time	14630.41	10	218.26	<0.0001
Treatments × Incubation time	11932.62	70	25.43	<0.0001
%MBC				
Treatments	170959.98	7	259.08	<0.0001
Incubation time	2165695.68	10	2297.40	<0.0001
Treatments × Incubation time	149650.97	70	22.68	<0.0001
%MBN				
Treatments	8185.52	7	107.54	<0.0001
Incubation time	56987.60	10	524.08	<0.0001
Treatments × Incubation time	5128.26	70	6.74	<0.0001
%N_{min}				
Treatments	180902.64	7	539.48	<0.0001
Incubation time	538260.26	10	1123.63	<0.0001
Treatments × Incubation time	65196.60	70	19.44	<0.0001

[a] Temporal dynamics in CH_4 fluxes was analyzed when all treatments emitted methane.

Table 3 Two-way ANOVA of the interaction between treatments and C/N ratios on the strength of residue-mixing effects for cumulative CO_2, CH_4 and N_2O emissions, GWP, DOC, DON, MBC, MBN and N_{min} concentrations at the end of the incubation. GWP, global warming potentials; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N_{min}, mineral nitrogen.

Sources	SS	df	*F*	*P*
%CO_2				
C/N ratio (R)	0.0126	1	29.66	0.0001
Treatment (T)	0.0097	1	22.75	0.0005
R × T	0.0000	1	0.01	0.9395
%CH_4				
C/N ratio (R)	0.1252	1	155.35	<0.0001
Treatment (T)	0.0524	1	65.01	<0.0001
R × T	0.1489	1	184.70	<0.0001
%N_2O				
C/N ratio (R)	0.0252	1	2.50	0.1395
Treatment (T)	0.0005	1	122.40	<0.0001
R × T	0.0223	1	108.25	<0.0001
%GWP				
C/N ratio (R)	0.0034	1	2.84	0.1179
Treatment (T)	0.0027	1	2.24	0.1602
R × T	0.0148	1	12.32	0.0043
%DOC				
C/N ratio (R)	0.0367	1	108.28	<0.0001
Treatment (T)	0.1204	1	355.40	<0.0001
R × T	0.0015	1	4.42	0.0573
%DON				
C/N ratio (R)	0.0003	1	0.06	0.00151
Treatment (T)	0.0122	1	2.47	<0.0001
R × T	0.1325	1	26.93	0.0766
%MBC				
C/N ratio (R)	0.0008	1	5.97	0.0491
Treatment (T)	0.0002	1	0.91	0.0048
R × T	0.0033	1	16.62	0.4441
%MBN				
C/N ratio (R)	0.0031	1	3.49	0.0862
Treatment (T)	0.0102	1	11.62	0.0052
R × T	0.0091	1	10.36	0.0074
%N_{min}				
C/N ratio (R)	0.0003	1	1.04	0.3278
Treatment (T)	0.0014	1	5.69	0.0344
R × T	0.0094	1	38.12	<0.0001

Figure legends

Fig. 1 Emissions of CO_2 (a), CH_4 (b) and N_2O (c) under different residue treatments. CK, control; C1, C2, C3 and C4, with equal amounts of C and at different C/N ratios; N1, N2, N3 and N4, with equal amounts of N and at different C/N ratios. The vertical bars represent standard error (n=3).

Fig. 2 Concentrations of soil DOC (a), DON (b), N_{min} (c), MBC (d) and MBN (e) under different residue treatments. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N_{min}, mineral nitrogen; CK, control; C1, C2, C3 and C4, with equal amounts of C and at different C/N ratios; N1, N2, N3 and N4, with equal amounts of N and at different C/N ratios. The vertical bars represent standard error (n=3).

Fig. 3 Dependence of CO_2 (a), CH_4 (b) and N_2O (c) emission fractions on residue C/N ratio. EF_C: CO_2, CH_4 or N_2O emission fraction in the equal C treatments; EF_N: CO_2, CH_4 or N_2O emission fraction in the equal N treatments.

Fig. 4 Dependence of DOC (a), DON (b), MBC (c), MBN (d) and N_{min} (e) on residue C/N ratio. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N_{min}, mineral nitrogen.

Fig. 5 Observed vs expected values of CO_2 (a), CH_4 (b), N_2O (c) fluxes, DOC (d), DON (e), MBC (f), MBN (g) and N_{min} (h) concentrations in the residue mixture treatments across the whole incubation time. Red symbols are indicative of statistically significant non-additive effects, and black symbols imply additive effects. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N_{min}, mineral nitrogen.

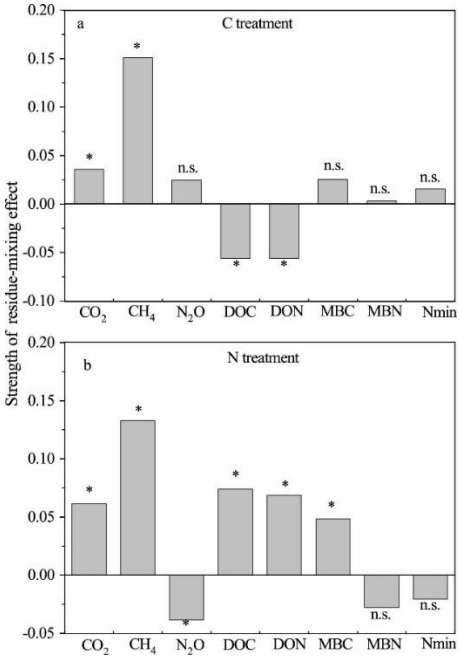

Fig. 6 Average values of the strength of residue-mixing effects for CO_2, CH_4 and N_2O fluxes, DOC,

DON, MBC, MBN and N_{min} concentrations in C (a) and N (b) treatments throughout the incubation period. DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N_{min}, mineral nitrogen. * indicates that the difference between zero and non-additive effect is significant (P<0.05); n.s. = no significant.

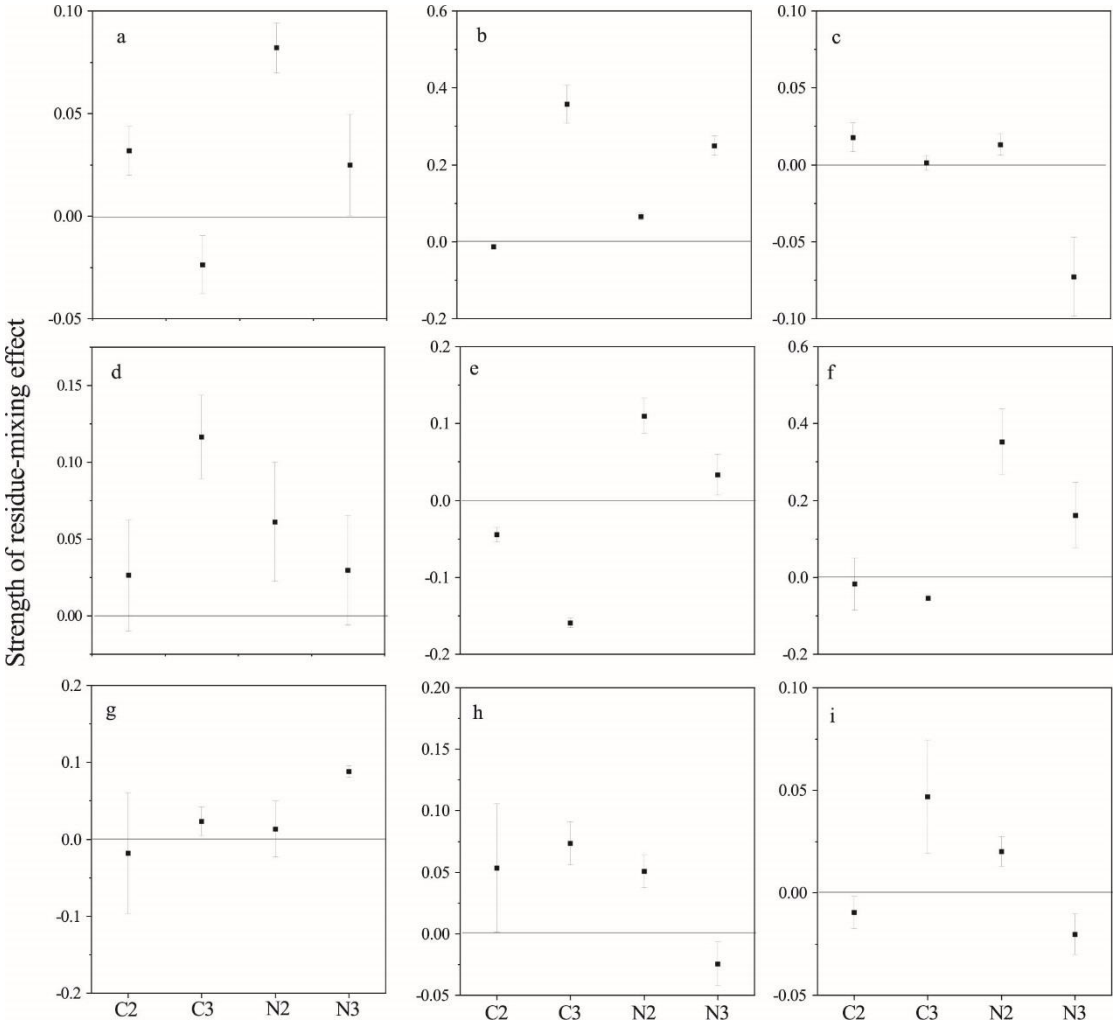

Fig. 7 Relationship between treatments and the strength of residue-mixing effects for cumulative CO_2 (a), CH_4 (b) and N_2O (c) emissions, GWP (d), DOC (e), DON (f), MBC (g), MBN (h) and N_{min} (i) concentrations at the end of the incubation. The point positively departs from zero level meaning synergistic effects, negatively departs from zero level meaning antagonistic effects. GWP, global warming potentials, DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N_{min}, mineral nitrogen.

Table S1 Changes in cumulative CO_2, CH_4 and N_2O emissions, DOC, DON, MBC, MBN and N_{min} concentrations under application residue and two-way ANOVA of the interaction between treatments and C/N ratios on cumulative CO_2, CH_4 and N_2O emissions, DOC, DON, MBC, MBN and N_{min} concentrations. The averages followed by the same letter in the same column are not significantly different (Duncan's test, $P < 0.05$), "-" indicates that CO_2, CH_4, N_2O, DOC, DON, MBC, MBN and N_{min} were not significantly affected by treatments, trial, or their interaction at the $P < 0.05$ level. DOC,

dissolved organic carbon; DON, dissolved organic nitrogen; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; N_{min}, mineral nitrogen

Treatment	C/N ratio	CO_2 (mg kg^{-1})	CH_4 (mg kg^{-1})	N_2O (μg kg^{-1})	GWP (mg CO_2 equivalents kg^{-1})	DOC (mg kg^{-1})	DON (mg kg^{-1})	MBC (mg kg^{-1})	MBN (mg kg^{-1})	N_{min} (mg kg^{-1})
Equal C	CK	874.54 c	2.70 c	46.85 a	956.04 d	108.81 d	25.17 d	430.90 d	64.66 cd	222.07 d
	C1	1175.82 a	25.35 b	39.03 b	1821.07 b	131.43 ab	44.17 a	476.19 a	73.84 a	279.04 a
	C2	1159.24 a	25.32 b	35.94 c	1802.83 b	126.60 bc	38.24 b	449.15 c	68.06 b	250.37 b
	C3	1075.18 b	33.72 a	35.74 c	1928.82 a	123.57 c	35.59 c	460.64 b	66.62 bc	241.80 c
	C4	1073.33 b	24.68 b	31.29 d	1699.65 c	133.90 a	35.41 c	460.61 b	62.74 d	213.77 e
Equal N	CK	874.54 e	2.70 e	46.85 a	956.04 e	108.81 e	25.17 c	430.90 c	64.66 b	222.07 d
	N1	1175.82 d	25.25 d	39.03 b	1821.07 d	131.43 d	44.17 b	476.19 b	73.84 a	279.04 a
	N2	1421.01 c	60.916 c	35.01 c	2954.35 c	144.50 c	45.23 ab	480.35 b	73.92 a	248.83 b
	N3	1674.67 b	111.25 b	29.14 d	4464.59 b	160.99 b	47.56 a	497.58 a	77.12 a	233.57 c
	N4	2279.25 a	219.62 a	25.53 e	777.43 a	174.61 a	45.97 ab	499.06 a	76.30 a	196.43 e
Two-way ANOVA	Treatment (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-<0.0001	-
	C/N ratio (R)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0193	-	-	<0.0001
	T×R	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	-	-	-