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CO_2 enrichment and N addition increase nutrient loss from decomposing leaf litter in subtropical model forest ecosystems

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As atmospheric CO_2 concentration increases, many experiments have been carried out to study effects of CO_2 enrichment on litter decomposition and nutrient release. However, the result is still uncertain. Meanwhile, the impact of CO_2 enrichment on nutrients other than N and P are far less studied. Using open-top chambers, we examined effects of elevated CO_2 and N addition on leaf litter decomposition and nutrient release in subtropical model forest ecosystems. We found that both elevated CO_2 and N addition increased nutrient (C, N, P, K, Ca, Mg and Zn) loss from the decomposing litter. The N, P, Ca and Zn loss was more than tripled in the chambers exposed to both elevated CO_2 and N addition than those in the control chambers after 21 months of treatment. The stimulation of nutrient loss under elevated CO_2 was associated with the increased soil moisture, the higher leaf litter quality and the greater soil acidity. Accelerated nutrient release under N addition was related to the higher leaf litter quality, the increased soil microbial biomass and the greater soil acidity. Our results imply that elevated CO_2 and N addition will increase nutrient cycling in subtropical China under the future global change.

Litter decomposition is a critical process in biogeochemical cycles in terrestrial ecosystems. Understanding litter decomposition process and its controlling factors are important for studying nutrient cycling. With increasing atmospheric CO_2, many experiments have been carried out to study the effect of elevated CO_2 on litter decomposition. However, over the last decade, there is considerable debate about the net effects of elevated CO_2 on this ecological process. Lower mass-loss[1–2], higher mass-loss[3–4] and no effect[5] of CO_2 enrichment on the leaf litter decomposition were reported. Atmospheric N deposition is a serious problem in some areas. Numerous studies have also been done to show the effect of N addition on litter decomposition. The stimulation of litter decomposition by N additions was shown in the studies of Torbert et al. (2000), Liu et al. (2006) and Mo et al. (2006)[6–8]. However, the inhibitory effect of N addition on litter decomposition was also detected by Tu et al. (2014) and Peng et al. (2014)[9–10]. The varied effects of elevated CO_2 and N addition on quality of litter, soil macroclimate environment, soil microbes etc. lead to different responses under different conditions[8–9,11–12].

Elevated CO_2 can affect litter quality by altering tissue concentrations of nutrients[13]. A decreased quality of litter under elevated CO_2 has long been considered the major mechanism decreasing litter decomposition[14–15]. A meta-analysis of data from naturally senesced leaves in field experiments showed that the N concentration in leaf litter was 7.1% lower in elevated CO_2 compared to that in ambient CO_2[16]. Different effects of elevated CO_2 on litter quality and decomposition have been documented[17]. However, most of research has been done in temperate areas[18], which are often N-limited and with low N deposition. Atmospheric N deposition is a serious problem in subtropical China. This led to high N deposition in precipitation in some forests (30–73 kg N $ha^{-1}yr^{-1}$)[8]. Recently, some studies have been done about the effects of N deposition on litter quality and nutrient mineralization in this area[8], while there is no report about the interactive effect of elevated CO_2 and N addition on the litter quality and decomposition in this N-rich subtropical area where N deposition will increase persistently in the future[19].

Elevated CO_2 has the potential to alter nutrient mineralization not only by changing the litter quality, but also by modifying forest-floor environmental conditions such as soil moisture and temperature[20–22]. Changes such as these in the forest environment would further affect the rates of biogeochemical process. Soil microbial processes are stimulated by soil humidity which accelerates litter decomposition and nutrient mineralization[12,23]. It was also reported that elevated CO_2 and N addition would change soil microbial biomass or community structure[24–25],

which would directly affect litter decomposition and nutrient loss. Hence, to know the mechanism of elevated CO_2 and N addition on the litter decomposition, soil macroclimate factors and soil microbial community structure should be monitored simultaneously.

We conducted a 21-month decomposition study from the 3rd to 4th year of the CO_2 fumigation and N addition. Our study was designed to investigate the effects of elevated CO_2 and N addition on the nutrient loss from the decomposing litter. The effects of elevated CO_2 and N addition on the initial litter quality, soil moisture and temperature as well as soil microbial community were also studied to show the major mechanisms affecting litter decomposition. We focused on changes in the quality and nutrient loss rates of litter from mixed leaf litter instead of individual species litter as natural forest ecosystems with mixed tree species distributes most area of subtropical China. We hypothesized that: (1) elevated CO_2 would decrease litter quality but increase soil moisture, and hence it would not affect litter decomposition rate in subtropical China; (2) N addition would increase litter quality but decrease soil microbial biomass, and it would not change litter decomposition and nutrient release either; (3) the interaction of elevated CO_2 and N addition would increase litter decomposition, and hence lead to more nutrient release.

Results

CO_2 and N effects on initial leaf litter quality. Initial C, N concentrations and C : N ratios in the leaf litter had no significant responses to increased CO_2 and N addition (Table 1). However, the average initial P concentrations in the CC, NN and CN treatments were 19.2%, 15.4% and 7.7% higher than CK, respectively (Table 1). The statistical analysis also showed the decreased ratios of C : P and N : P were found in the CC, NN and CN, when compared with CK.

Compared with the control, both elevated CO_2 and N addition increased significantly Ca and Mg concentrations in the leaf litter (Table 1). About 38.0%, 33.9% and 20.4% greater Ca concentrations in the leaf litter were shown in the NN, CN and CC treatments, respectively. About 33.3%, 33.3% and 8.3% greater Mg concentrations were detected in the NN, CN and CC treatments, respectively. Higher Al, Mn and Pb concentrations in leaf litter were also found in the chambers exposed to elevated CO_2 treatment.

CO_2 and N effects on soil pH, soil temperature and moisture. Both elevated CO_2 and N addition treatments significantly decreased soil pH in the 0–20 cm layer (Table 2). There was no treatment effect on soil temperature (Table 3). Soil moisture was significantly affected by the CO_2 treatment, N treatment and their interactions ($P < 0.001$ for all, Table 3). The greater soil moisture was found in the CC and CN chambers than CK. However, the N treatment decreased significantly the soil moisture, with the lowest soil moisture in the NN chambers (Fig. 1).

CO_2 and N effects on soil microbial properties. Abundances of PLFAs were used here as indicators of the active living biomass. The abundance of PLFAs for bacteria, fungal, gram-positive bacteria, and gram-negative bacteria were unaffected by elevated CO_2 in either the 0–10 cm or 10–20 cm soil layer (Fig. 2). However, N addition significantly increased ($P < 0.05$) the abundances of the total PLFAs and the PLFAs for total bacteria, gram-negative bacteria, AMF and SF PLFAs in the 0–10 cm soil layer (Fig. 2). The F : B ratio in the 10–20 cm soil layer was significantly higher in CC treatment.

CO_2 and N effects on leaf litter mass loss. Mass remaining was significantly affected by sampling time ($P < 0.01$) and the interactions of time and treatments ($P < 0.0001$). The significant differences between treatments occurred in Jan.2008 (about half a year), November 2008 (about 1.4 year) and Apr.2009 (about 1.8 year) ($P < 0.05$, Fig. 3). The decay rate constant (k) of litter decomposition was 0.711 for CK, 0.772 for NN, 0.831 for CC, and 1.076 for CN, with the significantly higher value in CN than in CK. At the end of the experiment, the average mass loss in the CN, NN and CC chambers were 45%, 37% and 18%, respectively, higher than CK chambers. Correlation analysis showed that decomposition coefficients (k) were negatively correlated ($R^2 = 0.602$, $P = 0.0399$) with the corresponding initial N : P ratio, however, the initial N and P concentration did not influence their decomposition coefficients.

CO_2 and N effects on nutrient loss during leaf litter decomposition. Carbon, N, P, Na, Ca, Mg, Mn released faster in the decomposing litter than other elements. Especially for Na, Mg and Mn, more than half of original weight released in two-month litter incubation. While the immobilization of K, Pb and Al from external sources was obvious as they showed relative high concentrations in the soil (Fig. 4). Except for Na and Mn, the other elements loss in the decomposing litter were all increased by elevated CO_2 treatment (Table 4, Fig. 4). The nutrient loss was following the order: CN > CC > NN > CK. Except for Na, Al and N, N addition also increased other element release from leaf litter. The interactive effects of elevated CO_2 and N addition only affected Ca, Mg and Al loss. The N, P, Ca and Zn loss were more than three times greater in the CN treatment than those in CK (Fig. 4). Statistical

Table 1 | Initial chemical composition of leaf litter used for the decomposition study under elevated CO_2 and N addition treatments. The treatments were: CK = control, NN = high N treatment, CC = elevated CO_2 concentration treatment and CN = elevated CO_2 concentration treatment + high N treatment. Values are means ± standard deviation. The different lowercase letters in the same row indicated significant treatment differences at $\alpha = 0.05$ level

	Treatments			
Concentration	CK	CC	CN	NN
C (mg g^{-1})	448.6(18.5)	441.3(20.6)	450.0(17.4)	442.0(18.8)
N (mg g^{-1})	12.4(0.5)	12.2(1.2)	11.6(0.6)	11.7(0.3)
P (mg g^{-1})	0.26(0.03) c	0.31(0.03) a	0.28(0.01) bc	0.30(0.00) ab
K (mg g^{-1})	0.62(0.04)	0.71(0.06)	0.74(0.15)	0.65(0.06)
Ca (mg g^{-1})	12.33(0.8) c	14.85(0.087) b	16.51(1.98) ab	17.02(0.73) a
Mg (mg g^{-1})	1.2(0.2) c	1.3(0.2) bc	1.6(0.2) ab	1.6(0.1) a
C:N ratio	36.31(1.87)	36.29(2.61)	39.04(1.95)	37.81(0.52)
N:P ratio	48.78(5.61) a	39.03(3.06) b	40.78(2.47) b	38.76(0.92) b
C:P ratio	1774(252) a	1414(125) b	1589 (82) b	1465(53) b
Al (mg g−1)	2.3(0.3) b	2.9(0.3) a	3.1(0.6) a	2.2(0.1) b
Mn (ppm)	314.2(53.8) b	409.3(50.6) a	368.2(63.7) ab	312.9(0.6) b
Pb (ppm)	20.3(2.7) b	26.3(6.5) a	27.5(3.2) a	22.7(0.2) ab
Zn (ppm)	218.7(79.5)	266.8(75.8)	288.3(46.5)	286.2(72.9)

Table 2 | Soil pH values in 0–20 cm soil layer from April 2007 to April 2009 under elevated CO_2 and N addition treatments. The treatments were: CK = control, NN = high N treatment, CC = elevated CO_2 concentration treatment and CN = elevated CO_2 concentration treatment + high N treatment. Values are means ± standard deviation. The different lowercase letters in the same row indicated significant treatment differences at $\alpha = 0.05$ level

	Treatments			
Time	CK	CC	CN	NN
Apr.2007	4.42(0.10)a	4.38 (0.02)ab	4.23(0.12)b	4.39(0.25)ab
Aug.2007	4.45(0.05)a	4.40(0.07)ab	4.31(0.10)b	4.39(0.22)ab
Nov.2007	4.46(0.11)a	4.39(0.04)ab	4.31(0.16)b	4.36(0.07)ab
Apr.2008	4.33(0.09)a	4.31(0.09)ab	4.22(0.09)b	4.28(0.05)ab
Aug.2008	4.43(0.05)a	4.38(0.06)ab	4.30(0.15)b	4.37(0.07)ab
Nov.2008	4.40(0.06)a	4.37(0.05)a	4.23(0.06)b	4.32(0.02)ab
Apr.2009	4.45(0.11)a	4.35(0.06)ab	4.21(0.11)c	4.34(0.05)b

analyses showed that increased P, Ca and Mg concentrations in the initial leaf litter were significantly related to nutrient loss.

Litter C : N ratios were not affected by CN, CC or NN. However, CO_2 enrichment increased N : P and C : P ratios significantly (Fig. 5). The ratios of N : P and C : P were also significantly affected by the interactive effects of CO_2 enrichment and N addition. However N addition alone did not change the ratios of N : P and C : P.

Discussion

The effects of CO_2 treatment on nutrient loss during leaf litter decomposition. Litter chemistry has been shown to be an important driver of litter decomposition in the tropics[28–29]. Although the litter quality parameters of N content, C : N ratio and lignin contents have been commonly recognized as important variables affecting litter decomposition rates, Mo et al. (2006), Zhang et al. (2008) and Waring (2012) indicated that P, Ca, Mg and K contents in litter were positively related to litter decomposition rates in tropical ecosystems[8,18,30], however, N was not an important factor which may due to the high N availability in this area[18]. Waring (2012) demonstrated that P concentration can explain 36% of the variance in foliar decay rates in tropical and subtropical forests[18]. It is commonly assumed that elevated CO_2 will reduce leaf litter quality[15–16]; however, the increased P, Ca and Mg concentrations in the initial leaf litter induced by elevated CO_2 were found in our experiment, which led to the greater litter decomposition and nutrient loss in our study. In a review, Kasurinen et al. (2007) also pointed out that litter P concentrations had generally increased under elevated CO_2[31]. The impact of CO_2 enrichment on nutrients other than N and P are far less studied. Cotrufo et al. (1998) reviewed pot seedling and growth chamber studies and did not find any clear CO_2 effects on K, Ca, Mg, Mn and Fe concentrations in tree litter[32].

Research showed that elevated CO_2 can reduce diffusive conductance and stomatal conductance of the leaves[33], which will lead to decreased rates of canopy transpiration and increased soil moisture in CO_2 enrichment plots[21,34]. Due to increased water availability soil microbial processes such as litter decomposition and nutrient mineralization were stimulated[12,23]. Increased soil moisture was found in the chambers exposed to elevated CO_2 treatment, which accelerated litter decomposition and nutrient loss in our study. Elevated CO_2 increased soil acidity in our study, which would increase cation leaching and also benefit nutrient release from decomposing litter.

Soil microbial community composition affects decomposition rates[12]. In our study, however, the abundance of PLFAs for bacteria, fungal, gram-positive bacteria, and gram-negative bacteria were all not affected by elevated CO_2 in either 0–10 or 10–20 cm soil layer, which suggests that the increased nutrient loss was not due to the increase of microbial biomass other than the increased microbial activity.

Higher litter decomposition rate and greater nutrient release in response to CO_2 enrichment were found in our study. This is not consistent with our hypothesis. Overall, nutrient loss during leaf litter decomposition induced by elevated CO_2 was due to increased leaf litter quality (increased P, Ca and Mg concentrations in the initial leaf litter), improved soil moisture and higher soil acidity in our study.

The effects of N addition on nutrient loss. In subtropical China, N was not a limited factor due to the high N availability in this area[8]. About 5.6 g N m^{-2} yr^{-1} wet N deposition was found in our study site[35]; hence N addition did not increase N concentration in the leaf litter in our experiment. N addition increased the nutrient loss from the decomposing leaf litter as CO_2 enrichment did. This was also in part due to the increased P, Ca and Mg concentration in leaf litter induced by high N addition. We also found that N addition decreased soil pH values and our published data showed about 6.3% and 3% added nitrogen was leached in 2006 in the CN and NN treatments, respectively[35], which led to the loss of cations to maintain an ionic balance and accelerated nutrient release from decomposing litter. The stimulation of litter decomposition by additions of N alone was also shown in the study of Torbert et al. (2000) and Liu et al. (2006)[6–7].

N enrichment is an element of global change that could influence the growth and abundance of many organisms[36]. With a meta-analysis, Treseder (2008)[36] showed that microbial biomass declined 15% on average under N fertilization and that declines in abundances of microbes and fungi were more evident in studies of longer durations and with higher total amounts of N added. However, N addition increased significantly the abundances of the total PLFAs and the PLFAs for total bacteria, gram-negative bacteria, AMF and SF in the 0–10 cm soil layer in our experiment. The higher microbial biomass in our experiment also led to the higher nutrient loss in the N addition treatment.

Table 3 | Effects of CO_2treatment (CO_2), N treatment (N), sampling season (Season) and their interactions on soil temperature and soil moisture. The probability values are shown in the table

Parameters	CO_2	N	CO_2*N	Season	CO_2* Season	N* Season	CO_2*N* Season	R^2
Soil temperature				<0.0001	0.0002			0.54
Soil moisture	<0.0001	<0.0001	0.0007	<0.0001	0.0006			0.38

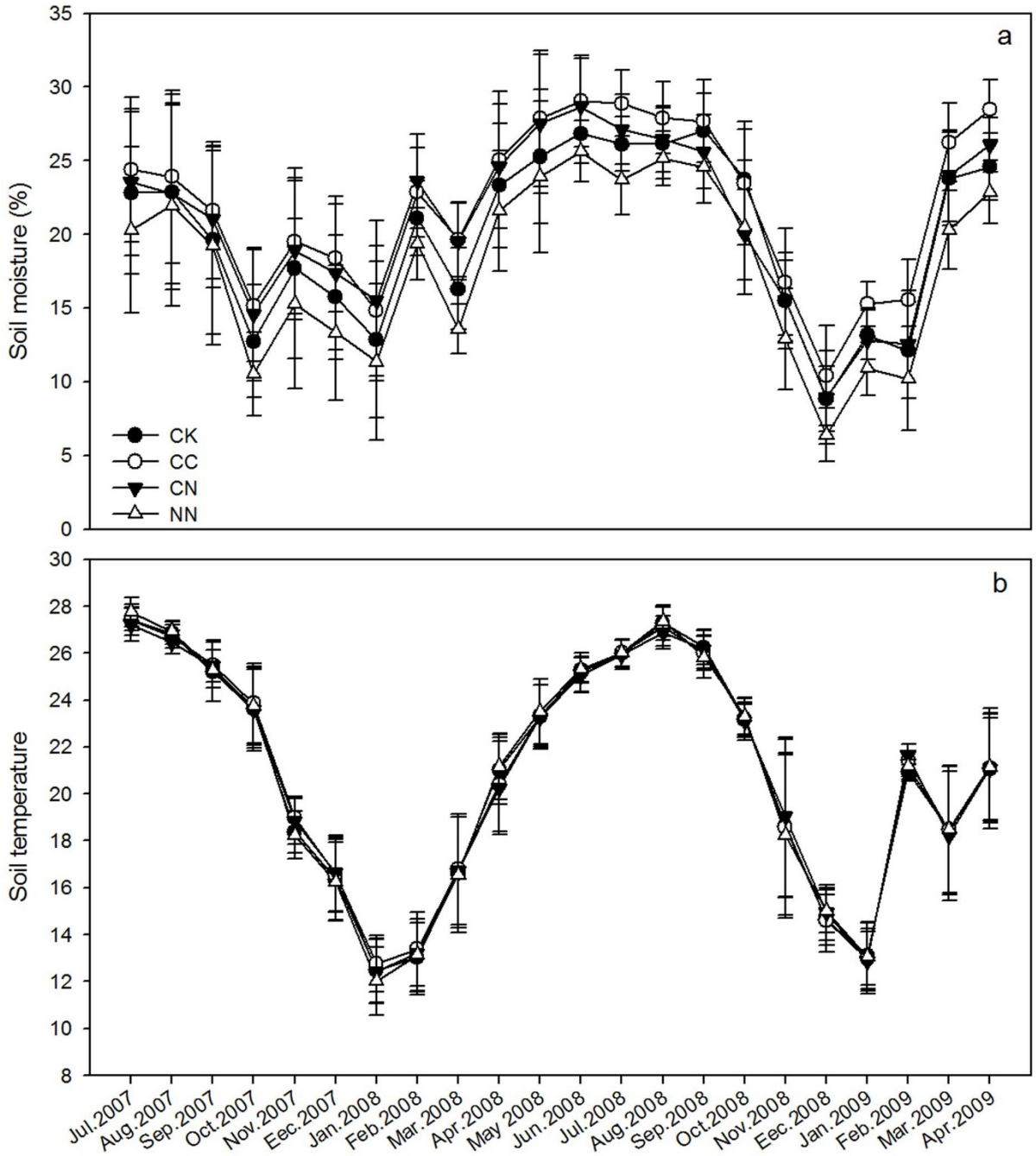

Figure 1 | Dynamics of soil moisture of the top 5 cm soil layer (a), and soil temperature at 5 cm below the soil surface (b) under different CO_2 and N treatments. The treatments are: CK = control, NN = high N, CC = elevated CO_2, CN = elevated CO_2 + high N. Data were cited from Deng et al. (2013)[26].

N addition increased leaf litter decomposition and subsequently nutrient loss, which is also not consistent with our hypothesis. More nutrient loss during leaf litter decomposition induced by N addition was due to increased leaf litter quality (increased P, Ca and Mg concentrations in the initial leaf litter), improved microbial biomass and higher soil acidity in our study.

The effects of the interaction of elevated CO_2 and N addition on nutrient loss. There are many experiments to show the effects of elevated CO_2 and N addition alone on the quality of leaf litter. A decreased quality of litter under elevated CO_2 has long been considered[16–17]. While an increased quality of litter under N addition has been mostly reported[8]. The study concerning the interactive effect of elevated CO_2 and N addition on the litter quality was few. In our experiment, we found that the interactive effects of elevated CO_2 and N addition improved significantly leaf litter quality, which led to the higher loss of elements from leaf litter decomposition process in the CN treatment.

Soil microbial processes are stimulated by soil humidity which accelerates litter decomposition and nutrient mineralization[12,23]. Although N addition alone decreased significantly the soil moisture ($p < 0.0001$), the interaction of elevated CO_2 and N addition increased soil moisture significantly ($p = 0.0007$), which also induced the higher nutrient loss form decomposing leaf litter when the chamber were exposed to both elevated CO_2 and N addition. Both elevated CO_2 and N treatment increased soil acidity in our study. Previous experiments demonstrated that high soil acidity would accelerate cation leaching[37]. In order to maintain an ionic

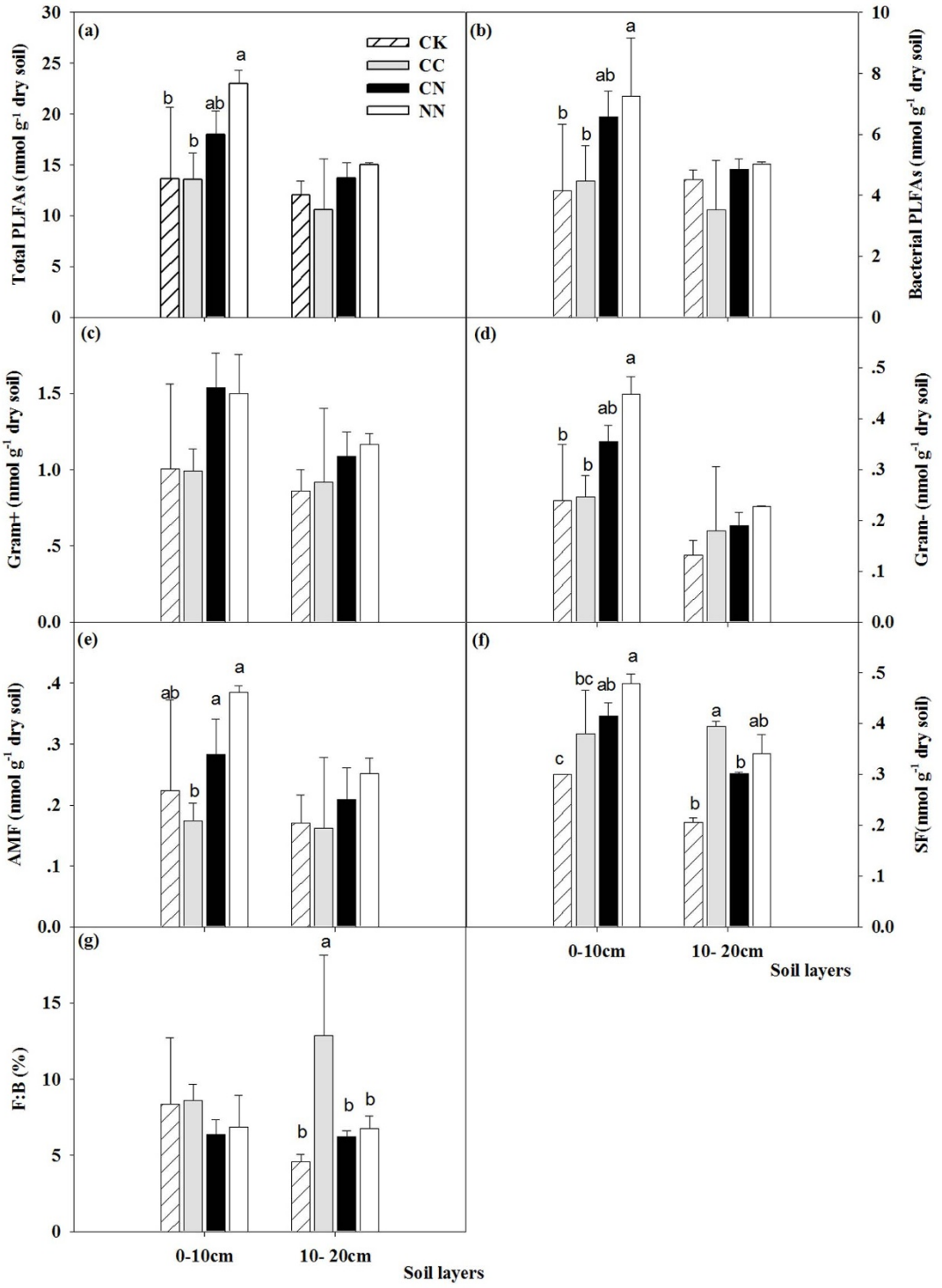

Figure 2 | Soil microbial PLFAs in different soil layers in 2009under elevated CO_2 and N addition treatments. (a) Total microorganisms (the sum of all the bacterial and fungal PLFAs), (b) Total bacteria, (c) gram-positive bacteria, (d) gram-negative bacteria, (e) arbuscularmycorrhizal fungi, (f) saprophytic fungi, and (g) the fungal to bacterial ratio. Bars indicate standard deviations of mean. In this figure, treatments are compared only within each soil layer and not between layers. The treatments are: CK = control, NN = high N, CC = elevated CO_2, CN = elevated CO_2 + high N.

balance, accelerated nutrient release from decomposing litter was then found in the treatment of both elevated CO_2 and N addition.

Overall, nutrient loss during leaf litter decomposition induced by the interaction of both elevated CO_2 and N addition was due to increased leaf litter quality (increased P, Ca and Mg concentrations in the initial leaf litter), improved soil moisture and higher soil acidity in our study.

Increased litter nutrient release under CO_2 enrichment and N addition will benefit subtropical forests in the future global change. In our study, we found higher litter decomposition and nutrient release induced by elevated CO_2, which is consistent with the previous reports[3–4]. In a certain time, higher litter decomposition indicates higher nutrient availability in soil[6], which will benefit tree growth. Nutrient limitation to forest primary productivity and other

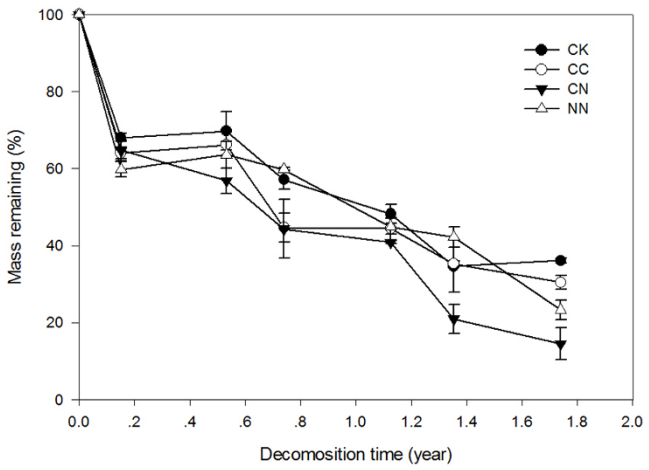

Figure 3 | Litter mass remaining (%) in decomposition litter under elevated CO_2 and N addition treatments. The treatments are: CK = control, NN = high N, CC = elevated CO_2, CN = elevated CO_2 + high N. Error bars are standard errors. Data were cited from Huang et al. (2014)[27].

ecosystem processes is widespread in tropical forests[38–39]. Nutrient available to plants in highly weathered tropical soils mainly depends on nutrient cycles in forest ecosystems[40]. Therefore, higher nutrient release induced by elevated CO_2 would increase nutrient cycles and benefit for subtropical forests under the future global change.

Higher litter decomposition and nutrient loss induced by N addition were found in our study. Mo et al. (2006) also showed that N addition increased significantly litter decomposition rates in disturbed and rehabilitated forests in subtropical China8. Higher nutrient loss from decomposing litter induced by N addition would also increase soil nutrient availability and benefit for the tree growth in the subtropical forests. However, as N deposition will increase persistently in the future in subtropical China[19] and the growth and abundance of many organisms will often be reduced with higher total amounts of N added[36], continuing monitoring should be done in the future study in this area.

Our Open-top chambers had a 0.7-m-deep belowground part. The part was delimited by a brick wall that prevented water exchange with soil outside the chamber. As tree seedlings in subtropical area grew very fast, we designed to add extra 600 mm water in each chamber every year. This might increase litter decomposition rates in all the chambers. However, we assumed that it won't affect the differences of elevated CO_2 and N addition treatments with the control on litter decomposition rates in our study.

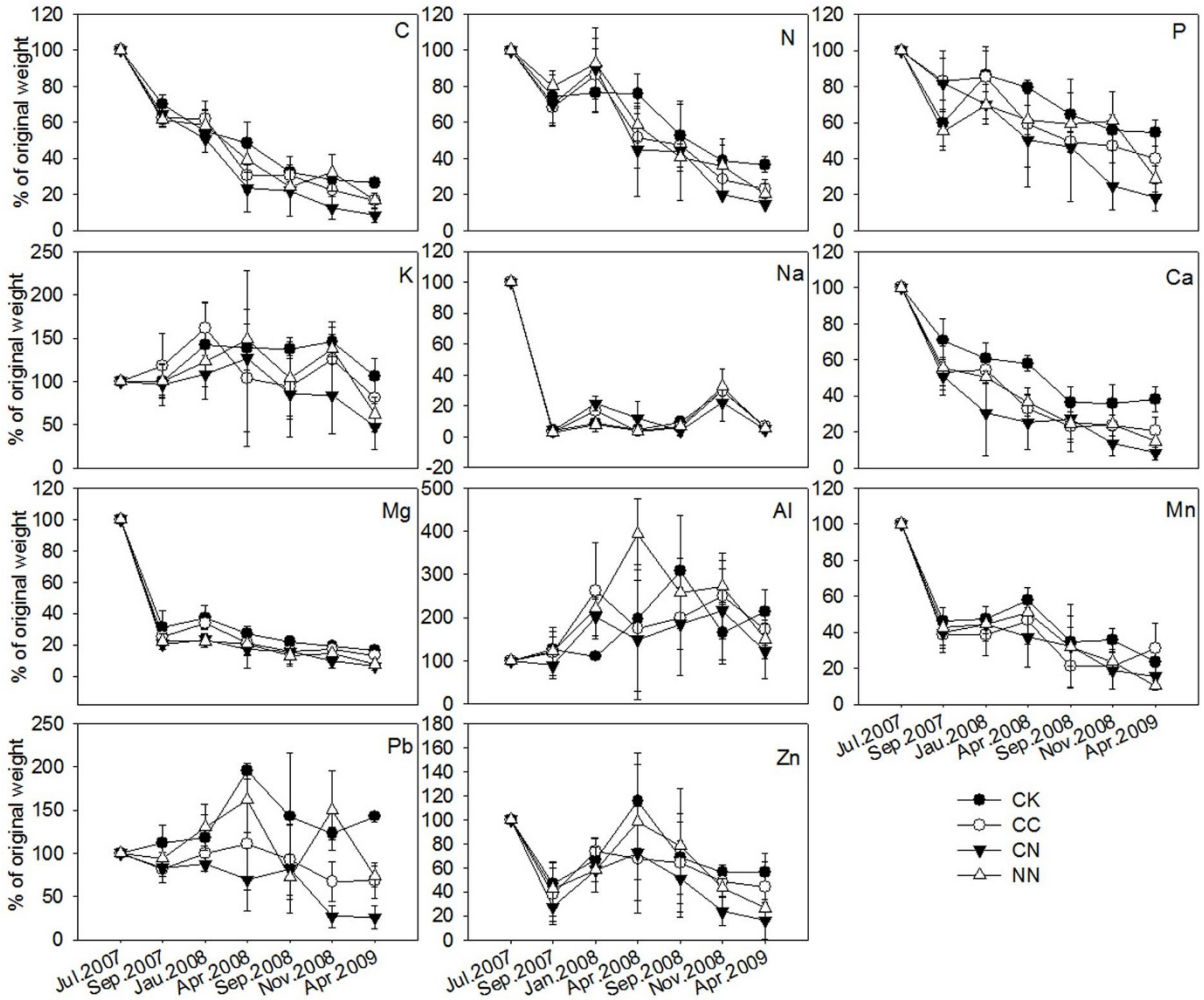

Figure 4 | Amounts of element remaining (as % of initial amount) in leaflitter residue during leaf litter decomposition process under elevated CO_2 and N addition treatments. Values >100% indicate net immobilization and values <100% net mineralization. The treatments are: CK = control, NN = high N, CC = elevated CO_2, CN = elevated CO_2 + high N.

Table 4 | Effects of CO_2 treatment (CO_2), N treatment (N), sampling time (Time) and their interactions on element release from decomposing leaf litter. The probability values are shown in the table

Parameters	CO_2	N	CO_2*N	Time	CO_2* Time	N* Time	CO_2*N*Time	R^2
K	0.04	0.002		<0.0001				0.40
Na				<0.0001	<0.0001		0.044	0.80
Ca	<0.0001	<0.0001	0.0013	<0.0001				0.77
Mg	0.0132	<0.0001	0.009	<0.0001				0.20
Al	0.0102		0.0162	<0.0001	0.0031		0.0219	0.49
Mn		0.044		<0.0001	0.0071	0.0105		0.58
Pb	<0.0001	<0.0001		<0.0001	<0.0001	0.0012	0.0068	0.67
Zn	0.0057	0.0024		<0.0001				0.45
C	<0.0001	0.0003		<0.0001	0.0005		0.0133	0.91
P	0.0091	0.0001		<0.0001	<0.0001			0.67
N	0.014			<0.0001		0.023		0.80

Methods

The study site and model forest ecosystem. The experimental site (23°10'46" N, 113°21'9" E) was located at the South China Botanical Garden in Guangzhou City, with an elevation of 126 m a.s.l. The site experiences a subtropical monsoon climate and has an average annual temperature of 21.9°C during the study period. July is the warmest, and January is the coolest month. The average annual rainfall during the study period was 1787 mm. More than 80% of the rain falls in the wet season (April-September), i.e., there is a distinct wet and dry season. The mean annual relative humidity of the ambient air is 78%. About 5.6 g N m^{-2} yr^{-1} wet N deposition was found in this study site[35].

In April 2005, a model forest ecosystem was established in each of 10 circular chambers with diameters of 3 m. The chamber system consisted of two parts. A 0.7-m-deep belowground part was enclosed by a brick wall that prevented water exchange with soil outside the chamber. All water discharged from the chamber was collected through three holes at the chamber base. A 3-m-high aboveground part was made from impermeable and transparent plastic sheets with an open top. Only 3% of the full sunlight was reflected or absorbed by the aboveground circular chamber wall. Soil at three depths (0–20, 20–40, and 40–70 cm) was collected from a nearby evergreen broad-leaved forest and used to fill the same depths of the belowground part of the chamber. The soil was a laterite with chemical properties shown in Table 5. One to two year old tree seedlings grown in a nursery were transplanted in the chambers with minimal damage to the roots. All the chambers were planted with 48 randomly located seedlings with 8 seedlings for each of the following 6 species: *Castanopsis hystrix*, *Syzygium hancei*, *Pinus massoniana*, *Schima superba*, *Acmena acuminatissima*, and *Ormosia pinnata*. These tree species are native and the most widely spread in Southern China. One tree for each species in each chamber was randomly harvested at the end of each year in the experiment to reduce crowding and to measure the tree biomass. As most seedlings of *Pinus massoniana* died in the second year of the experiment, only the leaf litter of other 5 species was considered in the study. For further details please see Liu et al. (2011, 2013)[41–42].

CO_2 enrichment and N addition. Treatments were applied starting in April 2005. A completely randomized design with two levels of CO_2 and two levels of N was used. Three chambers were enriched with CO_2 to achieve a concentration of 700 ppm inside the chamber's ambient air (treatment CC). Two chambers were treated by spraying seedlings with an NH_4NO_3 solution at an N addition rate of 10 g N m^{-2} yr^{-1} (treatment NN). Three chambers were treated with both elevated CO_2 and N addition (treatment CN). The remaining two chambers were used as controls and did not

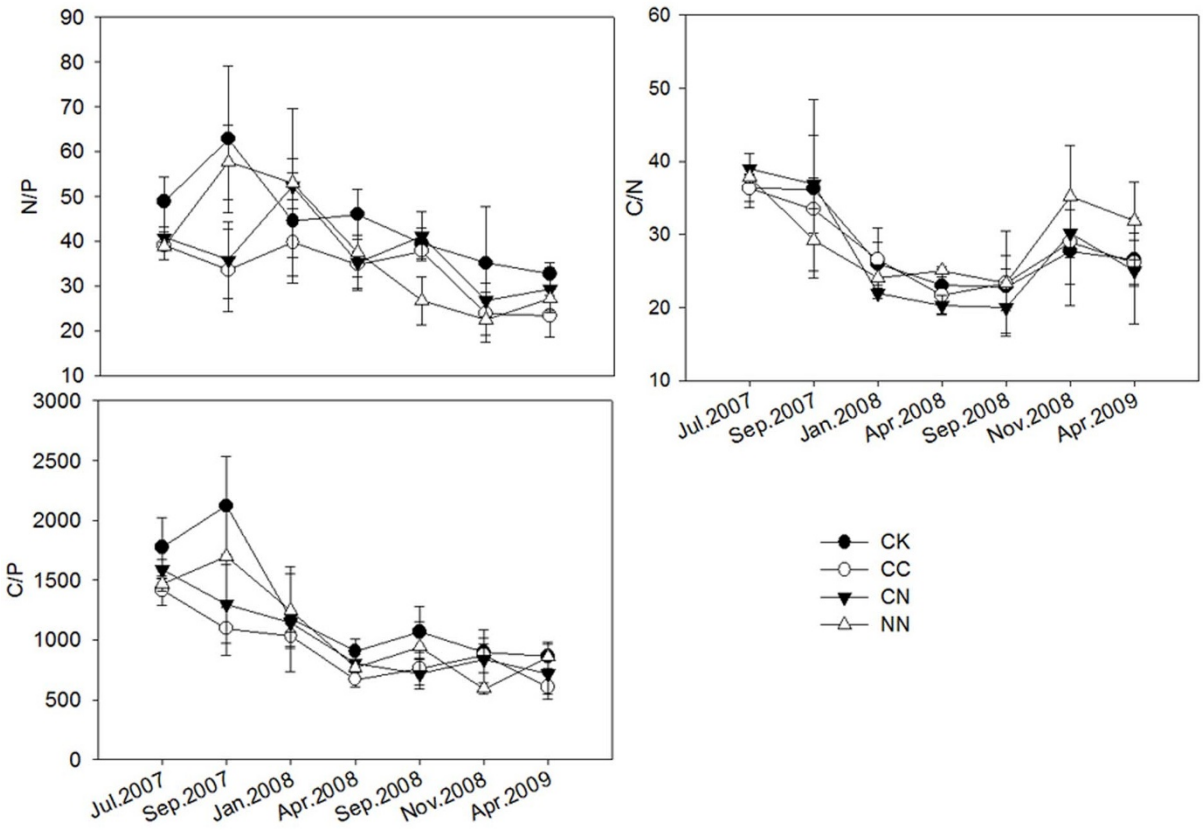

Figure 5 | Dynamics of N:P, C:N and C:P in the leaf litter residue during leaf litter decomposition process under elevated CO_2 and N addition treatments. The treatments are: CK = control, NN = high N, CC = elevated CO_2, CN = elevated CO_2 + high N.

Table 5 | The total concentrations of mineral elements in the initial soil. Standard errors are in brackets (n = 10). Unit for available P, Al, Cu and Mn is mg kg^{-1}, for others is g kg^{-1}. Data except Al, Cu and Mn were cited from Liu et al. (2008)[31]

Depth (cm)	pH	K	Na	Ca	Mg	P	Organic C	N	Al	Cu	Mn	Available P
0–20	4.15 (0.15)	6.30 (0.73)	0.64 (0.19)	1.03 (0.22)	1.03 (0.13)	0.30 (0.09)	16.33 (3.42)	0.52 (0.15)	1.77 (0.20)	4.69 (0.55)	78.70 (2.78)	2.13 (0.93)
20–40	4.27 (0.15)	5.03 (1.11)	0.63 (0.49)	0.57 (0.27)	0.84 (0.22)	0.18 (0.19)	7.78 (0.91)	0.36 (0.05)	1.55 (0.05)	4.68 (0.47)	73.68 (7.91)	0.42 (0.21)

receive the CO_2 enrichment or N addition (treatment CK); the ambient CO_2 concentration inside the CK chambers and NN chambers at mid-day ranged from 390 to 430 ppm during the experiment. All chambers had the same fan-generated wind speed and received 600 mm of extra tap water per year for irrigating the seedlings. The major element concentrations of the tap water were: K 0.68 mg L^{-1}, Na 0.33 mg L^{-1}, Ca 1.6 mg L^{-1}, Mg 0.77 mg L^{-1}, N 0.62 mg L^{-1}, P 0.001 mg L^{-1}, Fe 0.05 mg L^{-1}, Cu 0.01 mg L^{-1}, Mn 0.02 mg L^{-1} and Al 0.15 mg L^{-1}. The experiment was conducted for 5 years. Further details about the treatments and operation can be found in Liu et al. (2011, 2013)[41–42].

Soil pH, soil temperature and soil moisture. From April 2007 to April 2009, soil samples were collected from 0–20 cm layer. Soil pH was measured using a soil: water ratio at 1 : 2.5. From July 2007 to April 2009, soil temperature at 5 cm below the soil surface was monitored on five random locations within a treatment chamber with a thermocouple sensor once a week. Simultaneously, volumetric soil moisture of the top 5 cm soil layer was measured on five random locations within a treatment chamber using a PMKit[43].

Microbial community analysis. Soil was sampled by randomly collecting three cores of 2.5 cm diameter per chamber on 25 February 2009. The cores from each chamber were divided into 0–10 and 10–20 cm soil layers. The soil from each layer in each chamber was combined giving one composite sample. After stones and coarse roots were removed, each composite soil sample was passed through a 2-mm sieve and used for Phospholipid Fatty Acid (PLFA) analysis using the method described by Bossio et al. (1998)[44].

Peak areas (i.e., response values) were converted to nanomoles of PLFA per g of C using internal standards (19 : 0 nondecanoic methyl ester). Bacterial-specific PLFAs were i15 : 0, a15 : 0, i16 : 0, 16 : 1ω7c, i17 : 0, a17 : 0, 17 : 0, cy17 : 0, 18 : 1ω7c, and cy19 : 0[45–46]. The amount of 16 : 1ω7c and i15 : 0 can be used to estimate the abundance and relative abundance of gram-negative and Gram-positive bacteria, respectively[47]. The biomarker for arbuscular mycorrhizal fungi (AMF) was16 : 1ω5c[48]. The biomarker for saprophytic fungi (SF) was 18 : 2ω6, 9C[49]. The ratio of fungal PLFAs (sum of 16 : 1ω5c and 18 : 2ω6, 9C) to all bacterial PLFAs was used to indicate the ratio of fungal biomass to bacterial biomass (F : B)[45]. The biomarkers for actinomycetes were 10 Me 16 : 0, 10 Me 17 : 0, and 10 Me 18 : 0[50]. Other PLFAs (i14 : 0, 14 : 0, 15 : 0, 16 : 0, 16 : 0 2 OH, 18 : 1ω5c, 18 : 0, 16 : 1ω9c, 17 : 1ω8c, 18 : 1ω9c, and 18 : 3ω3c) were common to both bacteria and fungi. The amount of all PLFAs (sum of all lipids present, 20 or fewer carbons in length) was used as an index of living microbial biomass[45].

Leaf-litter decomposition and nutrient release. Naturally senesced mixed leaf litter was collected every month from March to June in 2007 in litter fall traps (0.3 × 0.3 × 0.1 m^3). Four litter fall traps were placed randomly in each chamber. The traps were made of plastic net that allowed throughfall to percolate easily but retained litter particles. The traps were located at a height of 10 cm aboveground. After removing understory litter and other woody material, leaf litter was aggregated within each chamber, air-dried and pooled across collection dates. A total of 15 g (ratios of oven-dried mass were determined by the proportion of air-dried mass of the litter fall after drying for 48 h at 70°C) of leaf litter was placed in 15 × 20 cm litterbags. Each litterbag had similar leaf litter composition. The mesh bags had a 1 mm mesh nylon top and a 0.2 mm mesh Dacron cloth bottom to reduce fragmented litter losses, and to allow microorganisms and small soil animal access. Litterbags were placed on the soil surface in the same chamber from which the litter was collected and left undisturbed until collection. Decomposition was followed for 621 days from July 2007 to April 2009. Two litterbags were retrieved on the following dates from each chamber: September 2007 (after 2 months), January 2008 (after 6 months), April 2008 (after 9 months), September 2008 (after 14 months), November 2008 (after 16 months) and April 2009 (after 21 months). At each removal, the litter samples were sorted to remove foreign material, weighed for mass loss after drying for 48 h at 70°C, and then finely ground for element concentration analysis.

Leaf litter chemical composition. Nutrient loss via the leaf litter composition, nutrient concentration in the initial leaf litter and the residual litter were determined. Carbon concentration was determined following the Walkley-Black's wet digestion method[51]. N concentration was measured using the Kjeldahl method[52]. Phosphorus concentration was measured photometrically after samples were digested with nitric acid. The concentrations of K, Ca, Mg, Al, Cu, Mn and Zn were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES; Optima-2000 DV, PerkinElmer, USA) after acid digestion.

Statistical analysis. The mass remaining of the leaf litter in each retrieved litterbag was expressed as a percentage of the initial dry weight of the leaf litter. The annual fractional weight loss is calculated using an exponential decay model[53] which is represented by the following equation: $X/X_0 = e^{-kt}$, where X/X_0 is fraction mass remaining at time t, X the remaining oven-dry weight at time t, X_0 the original oven-dry weight, "e" the base of natural logarithm, k the decomposition coefficient, and t the time.

Data analyses were carried out using the SAS (version 9.2, SAS Institute, Inc) software. Distributions that did not conform to homogeneity of variances or normality requirements were logarithmically transformed prior to analysis. ANCOVA was used to detect significant effects of CO_2 and N treatments on litter quality and its decomposition rate. When the effects were significant, they were further analyzed using Tukey multiple comparison test (HSD). Repeated measures ANOVA with Tukey's HSD test was used to examine treatment effects on soil pH, soil temperature and moisture as well as the element releasing rates during the litter decomposition process (including the main effects of CO_2 treatment, N addition, sampling time (season) and their interactions).

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Author contributions

J.L. analysed the data and wrote the manuscript. X.F. designed the study and proposed the scientific hypothesis. Q.D. did the measurement of soil temperature and moisture. T.H. did the microbial community analysis. W.H. did the statistical analysis. Y.L. prepared the figures.

Additional information

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