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Changes in CH_4 production during different stages of litter decomposition under inundation and N addition

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Abstract
Purpose Our aim was to examine linkages between mass loss, chemical transformation and CH_4 production during decomposition of leaf litters submerged under water. We hypothesised that (i) labile leaf litters would fuel a rapid, high rate of methane (CH_4) production and that recalcitrant litters would fuel long-lasting but lower emissions, (ii) leaf litters experiencing a greater alteration to chemical properties would stimulate increased CH_4 production and (iii) nitrogen (N) addition would increase CH_4 emissions.
Materials and methods Litters from six plant species were collected from a riparian ecosystem adjacent to Wyaralong Dam, located in Queensland, Australia, i.e., *Lophostemon confertus*, *Cynodon dactylon*, *Heteropogon contortus*, *Chamaecrista rotundifolia*, *Chrysocephalum apiculatum* and *Imperata cylindrica*. We evaluated the rate of mass loss and CH_4 emissions for 122 days of incubation in inundated microcosms with and without N addition. We quantified the chemical changes in the decomposing litters with ^{13}C-cross polarization and magic angle spinning (CPMAS) nuclear magnetic resonance (NMR) spectrum.
Results and discussion The inundation treatment of plant litters significantly affected decomposition rates. All litters decomposed in either inundated or aerobic microcosms were quite distinct with regard to the NMR spectra of their initial litters. N addition altered the NMR spectra under both inundation and aerobic conditions. The N treatment only marginally influenced the decomposition rates of *I. cylindrica* and *C. apiculatum* litters. The diurnal patterns of CH_4 production in the *H. contortus*, *C. rotundifolia* and *C. apiculatum* litters under inundation incubation could be expressed as one-humped curves, with the peak value dependent on litter species and N treatment. N addition stimulated CH_4 emission by *C. rotundifolia* and *C. apiculatum* litters and inhibited CH_4 emission from microcosms containing the litters of the three gramineous species, i.e., *I. cylindrica*, *C. dactylon* and *H. contortus*.
Conclusions Our results provide evidence that labile leaf litters could fuel a rapid, high rate of CH_4 production and that recalcitrant litters fuelled a lower CH_4 emission. We did not find that leaf litters with altered chemical properties stimulated increased CH_4 production. We also found that N addition was able to increase CH_4 emissions, but this effect was dependent on the litter species.

Keywords ^{13}C-CPMASNMR · CH_4 emission · Inundation · Litter decomposition · N addition

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1 Introduction

Methane is the third most abundant greenhouse gas after water vapour and CO_2, and its potential to induce global warming is 25 times greater than that of CO_2 (Crawford et al. 2016). In

general, methanogenic archaea can survive in an anoxic environment and can tolerate temporary aeration (Erkel et al. 2006; Høj et al. 2006). Leaf litters from riparian vegetation is a primary source of organic matter (OM) in freshwater ecosystems (Glazebrook and Robertson 1999). Submerged fresh litters can provide anoxic environment and substrates that regulate methanogenic activity and support CH_4 production (Zak et al. 2015).

Litter quality is commonly related to the chemical composition of plant litter, for example carbon/nitrogen (N) and lignin/N ratios and/or lignin and N content (Berg et al. 1993). This means that higher quality litter most often decomposes easily (Berg and McClaugherty 2008). Because litter OM of higher quality (e.g., lower C/N and lignin/N) is more readily decomposed than that rich in recalcitrant compounds, litters with more labile OM are readily available to microbial decomposers and might stimulate more methanogenic activity while submerged.

Litters contain different qualities of organic compounds in terms of structural and non-structural carbohydrates; thus, the potential for mass loss and CH_4 gas production varies from species to species. If different plant species have unique effects on CH_4 production, then evaluating traits of leaf litters has the potential to allow prediction of CH_4 emission flux. Leaf litters with higher decomposability can promote resource use efficiency in micro-decomposers (Freschet et al. 2012). Decomposability refers to variation in the rate of leaf decomposition associated with various chemical properties, e.g., the contents of N and labile and recalcitrant organic compounds (Hladyz et al. 2009). It has proven difficult to quantify litter decomposability in terms of organic chemical composition because litters are composed of a multitude of organic compounds. Recently, however, several papers have reported that the use of solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy with cross polarization and magic-angle spinning (^{13}C-CPMAS) can improve our ability to predict litter decomposition dynamics (Bonanomi et al. 2013). It is possible that identifying labile litters (i.e., with higher decomposability) by ^{13}C-CPMAS NMR among litters retained in freshwater ecosystems is more important than the identification of recalcitrant litters in understanding the effect of plant communities on CH_4 emissions.

Excess N fertilizer not taken up by crops can run off in storm water and increase N inputs to reservoir riparian ecosystems. The anthropogenic active N can directly influence freshwater ecosystem biota community structure and functioning (Gulis and Suberkropp 2003). Although numerous studies have examined the effects of N enrichment on primary productivity in unshaded, eutrophic aquatic ecosystems (Elser et al. 2007), less is known about how reactive N alters the function of ephemeral aquatic ecosystems that are primarily oligotrophic. Several studies have indicated that leaf litter decomposition and microbial activity in lentic ecosystems can be affected by the concentration of N in the water (Gulis and Suberkropp 2003; Gulis et al. 2004; Kuehn 2016). It is also known that micro-organisms colonizing the surface of submerged leaf litters can obtain N from water (Manning et al. 2015). High levels of excess N can potentially stimulate the immobilization of N into litter residues and may therefore influence decomposition rates and the availability of one- and two-carbon organic resources to methanogens (Irvine et al. 2012). Methanogenic bacteria are believed to be unable to gain energy from complex compounds (Whitman et al. 2014), as their growth depends on a limited set of one- or two-carbon compounds supplied by their associated anaerobic microbial communities. The effect of N addition on CH_4 emission associated with different litter species (or different litter qualities) while submerged remains unclear.

Our aim was to examine linkages between fresh leaf litter decomposition and CH_4 production under submergence. We assessed the rate of mass loss for 122 days, evaluated correlations between variations in decomposition rates with changes in leaf litter chemical composition and quantified the chemical changes in decomposing litters with the ^{13}C-CPMAS NMR spectrum. Our hypothesis was that labile leaf litters would fuel a rapid and high rate of methane production and that recalcitrant litter would fuel long-lasting but lower emissions. We further hypothesised that leaf litters that have undergone greater changes in chemical composition would stimulate increased CH_4 production. Additionally, we hypothesised that N addition might increase CH_4 emissions.

2 Methods

2.1 Sampling site description

We collected the fresh leaf litters of 6 plant species from the riparian area within a dam-watershed ecosystem (27° 56′S, 152° 51′E) at Wyaralong Dam, located in Queensland, Australia. This site is situated on sandy loam soil and was inundated due to construction of the dam in January 2011. These six species were chosen to represent the most common plant functional types present in the ecosystem. Soil at the riparian site is a brown kurosol, and the site slope is 6–24%. The annual rainfall is approximately 900 mm at the Wyaralong Dam region. Since 2007, the mean minimum temperature was 13.1 °C, and the mean maximum temperature was 26.5 °C.

2.2 Sampling, processing and incubation

All leaf litters included in this study were collected from the riparian area within a dam-watershed ecosystem (27°56′S, 152°51′E), Wyaralong Dam, located in the Queensland, Australia. The litters were from a dominant tree species

(*Lophostemon confertus* (Br.) Wilson & Waterh), a short grass species with high N content (*Cynodon dactylon* (L.) Pers.), a tall grass species with low N (*Heteropogon contortus* (L.) Beauv. ex Roem. & Schult.), a legume species (*Chamaecristarotundifolia* (Pers.) Greene), a sage brush species (*Chrysocephalum apiculatum* (Labill.) Steetz) and *Imperata cylindrica* (Table 1). These species are distributed throughout the riparian zone of the Wyaralong Dam reservoir.

Approximately 2.0 g of litters (dry weight) and 500 ml of reservoir water were placed inside 1000 ml gas-tight glass Mason jars fitted with lids (S, inundation regime). The reservoir water collected from the Wyaralong Dam was filtered through 100 mesh nylon mesh prior to incubation. Total N content in the water was 0.32 mg L^{-1}, total organic C was 5.33 mg L^{-1} and pH was 8.85. Nitrogen treatments included a low N treatment (1 mg L^{-1} N-NH_4NO_3) and a high N treatment (3 mg L^{-1} N-NH_4NO_3). The litters were also incubated at 60% moisture (M, moisture regime) as a control with low N (N added in an amount equal to the lower N treatment above) and without additional N. The reservoir water was used to manipulate the litter moisture at first time, and after that purified water was used during the incubation process. Therefore, there were five treatments in this study, i.e., litter inundated with low and high N addition (1 mg L^{-1}, S+N; 3 mg L^{-1}, S+3N) and without N addition (S), and litter moisturized with N (M+N) and without N addition (M). Three replicates were produced for each N treatment and each plant species. The jars were incubated in the dark at 24 °C. After a 4-h equilibration period at 24 °C, the jars were capped tight with a lid, and CH_4 accumulation in the headspace of each jar was collected at 0- and 2-h periods.

The jars were covered with a cap loosely to allow air flow and were uncovered manually every 12 h for a 5-min ventilation period during incubation, except when coinciding with gas collection time points. The weight of the jars was periodically measured to determine water content. The jars were gently shaken by the hand every 12 h, and water was added based on the weight measurement to keep the head space constant. At 0.167, 1, 2, 4, 6, 9, 12, 16, 20, 27, 35, 51, 75, 89 and 122 days of incubation, the jars were sealed with a gas-tight lid equipped with a rubber septum and the gas in the jar was collected three times using a 10-ml syringe at 1-h intervals. Before sealing the jar and beginning the gas collection, the jars were shaken by the hand for 30 s to release the soluble CH_4. The rate of CH_4 production in each incubator was determined by measuring the increase in CH_4 concentration in the headspace. The incremental production rate was calculated from the differences in CH_4 concentration over time and was corrected for headspace volume and atmospheric pressure and temperature differences among sampling time points. The concentrations of CH_4 were determined using a gas chromatograph equipped with a flame ionization detector (FID) (Shimadzu GC-2010, Japan) and packed with a column (Q80-100 mesh). The CH_4 emission was calculated using the following function:

$$f_{\mathrm{em}} = \rho \times V \Big/ W \times \Delta \mathrm{C} \Big/ \Delta \mathrm{t} \times 273 \Big/ T$$

where ρ (g m^{-3}) is the density of CH_4 at 0 °C, V (m^3) and W (kg) are the head space volume of the jar and the initial litter dry weight, respectively, ΔC is the change in CH_4 concentration (ppm) over the measurement period Δt (h) and T is the absolute temperature.

2.3 Litter chemistry analysis

The residual litters in each jar were dried at 60 °C for 48 h. After weighting, the litter residues were finely ground and used for the NMR analyses. The litter decomposition rate was determined after 120 days incubation and calculated as the proportion of the initial mass that remained. To determine the chemical composition of litters before and after decomposition, we characterized the chemical shifts in organic C functional groups in the powdered litter samples using solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross polarization and magic-angle spinning (CPMAS NMR) (Zheng et al. 2014). NMR spectroscopy is one of the most powerful non-destructive methods available for plant litter chemical structure studies, providing detailed compositional information on complex matrices, such as plant leaf litters. The CPMAS-NMR data were obtained using a Varian OXFORD 300 spectrometer (Varian Inc., CA) operating at a frequency of 75.4 MHz. Samples were packed in a silicon nitride rotor (OD = 7 mm) and spun at 5000 Hz at the magic angle. The cross polarization sequence tancpx contained within the rnmrJ 3.1A software package was used. The contact time was 1.2 ms, acquisition time was 20 ms and the recycle delay was 2.5 s. A Lorentzian line broadening function of 20 Hz was applied to all spectra, and 2000 transients were collected for litter samples using a sweep width of 36 kHz. The ^{13}C chemical shift values were referenced relative to tetramethylsilane at 0 ppm. The MestReNova package was used to estimate the relative integrated areas of eight regions between 0 and 210 ppm. The eight regions were alkyl (0–45 ppm), methoxyl (45–60 ppm), carbohydrate (60–90 ppm), di-O-alkyl (90–110 ppm), aryl (110–140 ppm), phenolic (140–160 ppm), carboxyl (160–190 ppm) and ketone (190–210 ppm).

2.4 Statistical analyses

The differences in mass loss between the control and N addition treatment under inundation were analysed using one-way ANOVA. The effects of N addition on mass loss in the moisture regime microcosms were analysed by t test. One-way ANOVA was used to test the effects of N addition on the

Table 1 Summary of initial plant litter properties ($n = 4$ instrumental replicates)

	C%	N%	Lignin%	C/N	Lignin/N
Imperata cylindrica	45.37 (0.07)	0.66 (0.01)	11.76 (0.29)	68.30	17.82
Lophostemon confertus	49.19 (0.54)	1.02 (0.02)	27.00 (0.64)	48.11	26.47
Chamaecrista rotundifolia	46.79 (0.78)	2.51 (0.06)	12.89 (0.82)	18.66	5.14
Cynodon dactylon	41.29 (0.40)	1.23 (0.02)	7.69 (0.68)	33.45	6.25
Chrysocephalum apiculatum	45.37 (0.3)	1.01 (0.02)	13.05 (0.54)	44.79	12.92
Heteropogon contortus	45.80 (0.23)	0.75 (0.01)	11.16 (0.36)	60.69	14.88

accumulative emission of CH_4. The Tukey honest significant difference test was used to compare the means. Minitab Statistical Software (version 17.0, Minitab, Inc., State College, Pa.) was used to run these statistical analysis, using a significance level of $P = 0.05$. To visualize the differences in litter quality among litter types and their response to N addition, we performed non-metric multidimensional scaling (NMDS) using Bray-Curtis dissimilarity matrices on the eight NMR spectra regions. The chemical shift data were also analysed using NMDS with PC-ORD version 5. The relative amount of C components from the ^{13}C-CPMAS NMR spectra were analysed using PCA to assess the effect of N addition on the litters from each plant species. The PCA statistical analysis was performed using Canoco 5.0 (Šmilauer and Lepš 2014; ter Braak and Šmilauer 2015).

3 Results

3.1 Initial chemistry of litters

The initial C, N and lignin concentrations of the studied litters differed among the six species (Table 1). *L. confertus* litters had a different chemical profile compared to the other five litters, while *I. cylindrical* litters were similar to those of *H. contortus* (Table 1). *C. rotundifolia* leaf litter had the highest concentration of nitrogen (N); while *H. contortus* had the lowest N concentration (Table 1). The lignin content was higher for *L. confertus* but lower for *C. dactylon*.

3.2 Mass loss during litter decomposition under inundation

During the 122 days of incubation while immersed in reservoir water, the leaves lost 21–68% of their initial mass. *C dactylon* leaves decomposed nearly three times more rapidly than *I. cylindrica* leaves (Table 2). The inundation treatment of litters affected the decomposition rates significantly (Tables 2 and 3). This effect was dependent on litter species (Table 3). The decomposition rates of *C. rotundifolia*, *C. dactylon*, *C. apiculatum* and *H. contortus* were higher under inundation than in the moisture incubation treatment, but the reverse effect was found for *L. confertus* litters. The N treatment only marginally influenced the decomposition rate in some litters, e.g., *I. cylindrica* and *C. apiculatum.*

3.3 Litter chemical components during litter decomposition under inundated conditions

The first two PCA axes explained more than 90% of the variation in the chemical changes in the litters (Fig. 1). Principal components axis 1, which explained more than 90% of the variability for *C. rotundifolia*, *C. apiculatum*, *C dactylon*, separated the inundated litter residues from the litters in the moisture regime treatment. This axis was correlated with O-alkyl and aryl. Litters from all species decomposed in either inundated or moisture microcosms were quite distinct with regard to the NMR spectra of their initial litters. PCA of the NMR spectra showed separation of litters without N addition from that with N addition, except for *C. apiculatum*. In addition, inundated litters of *C. dactylon* showed a similar NMR spectra without N and with the low N addition treatment. We observed that N addition altered the NMR spectra under both inundation and aerobic treatments. Component 1 loadings showed that *C. rotundifolia*, *I. cylindrica* and *C. dactylon* litters with N addition and water treatments were distinguished by O-alkyl-C, *I. cylindrica* litters by aryl-C, *H. contortus* by carboxyl-C and *L. confertus* litters by ketones; and component 2 loadings showed that N and incubation effects on *C. rotundifolia* and *I. cylindrica* litters were separated by carboxyl-C, *C. dactylon* and *H. contortus* litters by alkyl-C, *L. confertus* litters by O-alkyl-C and *C. apiculatum* litters by methoxyl-C, respectively.

Bray-Curtis dissimilarity between initial litters and litters after 122 days of incubation can explain 93% of the variation in mass loss under the moisture regime treatment, whereas only 30% was explained under the inundation regime treatment (Fig. 2). According to the litter NMR spectra, all of the litters studied can be grouped into three classes: class I, *C. apiculatum* and *C. rotundifolia*; class II, *I. cylindrica*, *C. dactylon* and *H. contortus*; and class III, *L. confertus* (Fig. 3).

Table 2 Decomposition rate (means ± SE) after 122 days of incubation. Litter decomposition rate (%) was determined after 120 days incubation and calculated as the proportion of the initial mass that remained

Species	Inundation			Non-inundation	
	CK	N	3N	CK	N
I. cylindrica	21.93(0.68)$^{b}\alpha$	22.82(0.34)$^{ab}\alpha$	24.30 (0.39)$^{a}\alpha$	24.45 (2.19)β	21.41 (2.76)αβ
L. confertus	25.44 (4.87)αβ	28.25 (3.50)αβ	25.73 (2.64)α	31.16 (1.94)γ	25.34 (3.65)αβ
C. rotundifolia	62.14 (1.08)$^{A}\delta$	61.44 (0.35)$^{A}\delta$	62.31 (0.69)$^{A}\delta$	49.00 (1.38)$^{B}\varepsilon$	49.00 (0.96)$^{B}\gamma$
C. dactylon	67.35 (1.32)$^{A}\delta$	68.40 (2.28)$^{A}\varepsilon$	66.30 (2.93)$^{A}\delta$	42.07 (1.15)$^{B}\delta$	46.55 (2.59)$^{B}\gamma$
C. apiculatum	42.55 (1.94)$^{bB}\gamma$	45.30 (1.55)$^{abB}\gamma$	50.46 (1.30)$^{aA}\gamma$	28.79 (0.60)$^{C}\beta\gamma$	27.93 (1.50)$^{C}\beta$
H. contortus	32.13 (0.71)$^{A}\beta$	33.04 (1.25)$^{A}\beta$	32.24 (0.97)$^{A}\beta$	18.26 (1.49)$^{B}\alpha$	19.62 (0.93)$^{B}\alpha$

Note: Capital letters indicate differences in decomposition rates of litter across treatments, lowercase letters refer to differences under the inundation regime, whereas Greek small letters indicate differences among litters under each treatment

3.4 CH_4 production during litter decomposition under inundation

The diurnal patterns of CH_4 production in *H. contortus*, *C. rotundifolia* and *C. apiculatum* litters while incubated under inundation can be expressed as one-humped curves (Fig. 4). The peak value appeared to be dependent on litter species and N treatment. In this study, no significant CH_4 production from the litters under the moisture regime treatment was detected, regardless of the addition of N. The detectable significant emission of CH_4 from *L. confertus* litter inundation jars was found to fall behind that of *I. cylindrica*, *H. contortus*, *C. rotundifolia* and *C. apiculatum* jars, in which the former peaked at 30 days and the latter litters at approximately 16 days. The production of CH_4 from *C. dactylon*, *H. contortus*, *C. rotundifolia* and *C. apiculatum* litters was higher than that of *I. cylindrical* and *L. confertus* litters (Fig. 5). N addition in *H. contortus* and *I. cylindrica* microcosms suppressed the accumulative production of CH_4, whereas a significant stimulation effect was observed in the *C. rotundifolia* and *C. apiculatum* microcosms. No significant relationship but a weak positive trend was shown between mass loss and cumulative CH_4 production (Fig. 6a). We found that cumulative CH_4 production was related to the Bray-Curtis distance of NMR chemical properties between initial litters and decomposed litters under the lower N addition regime (Fig. 6b).

4 Discussion

4.1 Effect of inundation on mass loss

Numerous studies have demonstrated that litter quality controls its rate of decomposition under similar environmental conditions (Melillo et al. 1982; Trofymow et al. 2002), e.g., microcosms in our study. Litter quality is often defined as the concentration of N and lignin in litters. High quality litters with higher N and lower lignin content decompose faster than litter with higher lignin and lower N (Xu et al. 1992). The reservoir riparian plant species considered in our study were

Table 3 Summary of the generalized linear models (GLMs) for the effects of water regime, litter species and N addition on litter mass loss

	DF	SS	MS	*F*	*P*
LitterID	5	18,293	3659	310.601	<2e−16***
Water regime	1	2581	2581	219.130	<2e−16***
N addition	2	14	7	0.578	0.564
LitterID × water regime	5	1738	348	29.515	5.42e−15***
LitterID × N addition	10	128	13	1.083	0.390
Streatment × N addition	1	17	17	1.426	0.237
LitterID × water regime × N addition	5	70	14	1.183	0.328
Residuals	60	707	12		

DF degree of freedom, *SS* sum of squares, *MS* mean square

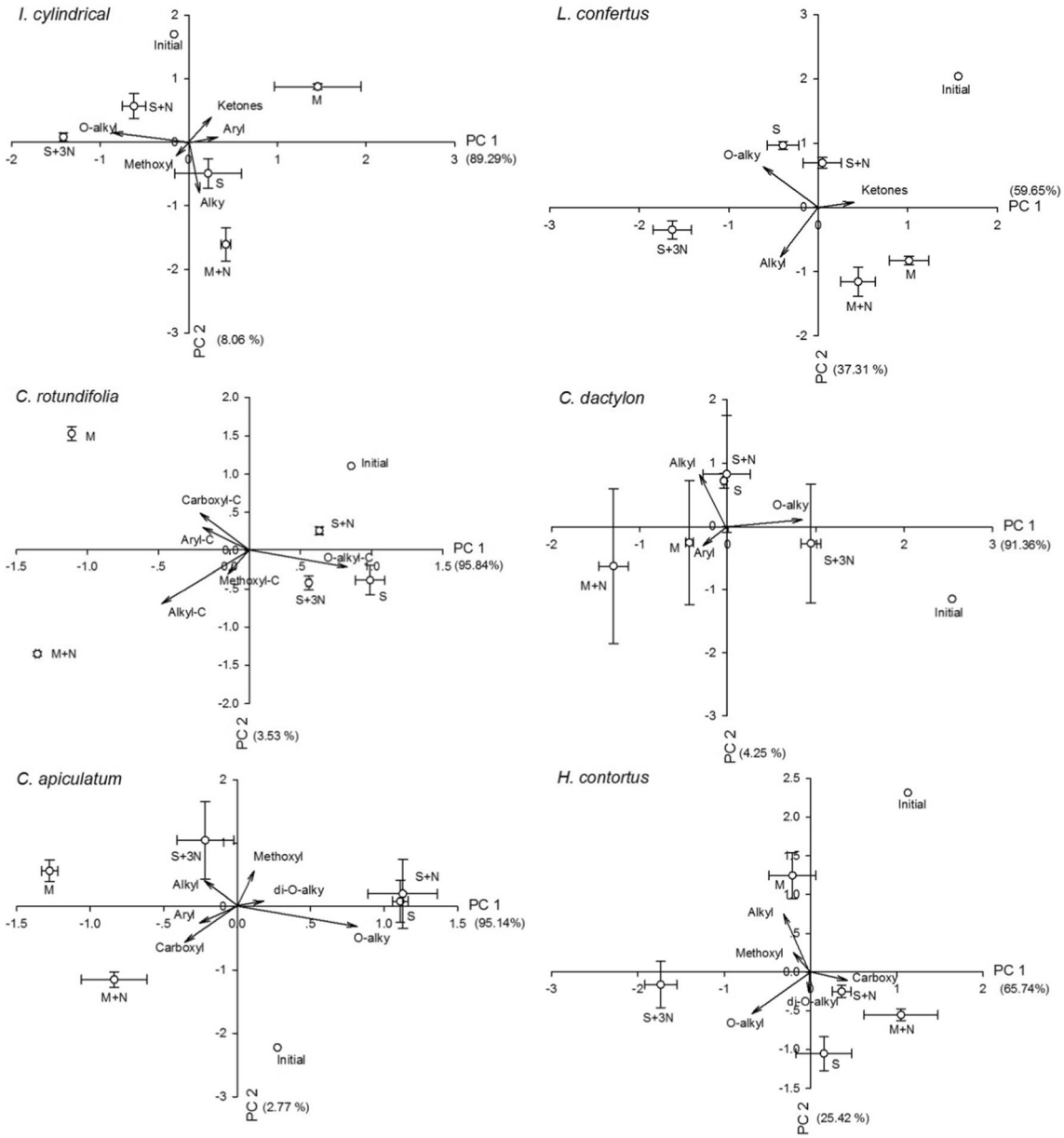

Fig. 1 Principal component analysis of the relative intensities of eight ^{13}C-CPMAS NMR regions of litters from different treatments and initial status. The values in parentheses on each axis label show the amount of variation in the dataset explained by the PCA axis. *Graphed values* represent means (n = 3). *S* inundation regime without N addition, *S+N* inundation regime with N addition of 1 mg L^{-1}, *S+3N* inundation regime with N addition of 3 mg L^{-1}, *M* litter moisturized without N and *M+N* moisturized with N addition

I. cylindrica, *L. confertus*, *C. rotundifolia*, *C. dactylon*, *C. apiculatum* and *H. contortus*. According to the litter NMR spectra, the litters from these species can be grouped into three classes: class I, *C. apiculatum* and *C. rotundifolia*; class II, *I. cylindrical*, *C. dactylon* and *H. contortus*; and class III, *L. confertus*. These litters can also be characterized by different qualities, *C. rotundifolia* displayed the highest quality and decomposed the fastest (high decomposability) while *L. confertus* had the lowest quality and decayed the slowest (low decomposability). The significant differences in mass loss between these six types of litters are likely explained by their quality. In the flooding seasons, these riparian species are generally inundated as the reservoir impounds. The senescent leaf litters of these plant species, therefore, decompose in an aquatic habitat that differs from drier terrestrial habitats. The decomposition rate of these litters and their residue chemistry are expected to change with flooding events. Indeed, we found that mass loss and chemical composition of the litters that decomposed under flooded conditions differed markedly from the litters in the moisture regime microcosms.

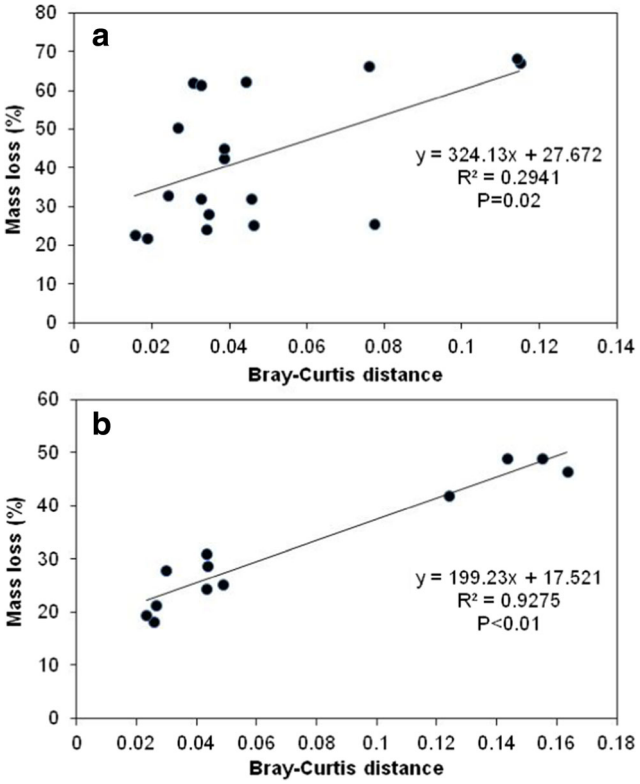

Fig. 2 Relationships between litter mass loss and Bray-Curtis distance of NMR chemical properties under **a** inundation treatment and **b** moisture treatment

4.2 Effect of N addition on mass loss

We also observed that N addition resulted in a significant increase in mass loss from *I. cylindrica* and *C. apiculatum* litters under the inundation treatment. The litters of both species are lower in quality compared to *C. rotundifolia* litters, which have higher N content and a low lignin/N ratio. No significant response to N addition was found for any of the litters studied under the moisture regime. These results are in general agreement with a few previous studies that showed little or no response of litter mass loss to N addition (Knorr et al. 2005). The stimulation effects of higher N addition on decomposition in *I. cylindrica* and *C. apiculatum* litters suggest that litter decomposition in both species is limited by N. No effect of N addition was observed for the higher quality litters of *C. rotundifolia* and *C. dactylon*, likely due to the limitation of other nutrients (e.g., phosphorus).

4.3 Effect of N addition and inundation on litter chemical components during decomposition

Our results demonstrated that N addition can modify the chemical composition of litter residues without directly affecting litter mass loss. The decoupling of litter residue chemistry and mass loss during decomposition may have implications for soil organic matter formation and stability. In addition, litter input quantities and decomposition rates have been used to make predictions of future ecosystem C dynamics (Liski et al. 2005; Cotrufo et al. 2013). Incorporation of N-modified litter residue materials into soil organic layers provides an important C retention mechanism for ecosystems. Considering the chemical modification of litter residues by N addition could help to improve understanding of soil C pool formation and stability.

We observed significant shifts in the chemical composition of litter organic C with N addition in both of the water regimes (Figs. 3 and 5). However, the nature of these shifts varied for the six types of litters we studied. One possible explanation could be that N addition altered micro-decomposer community composition and activities, thereby modifying the chemistry of litter residues. We found that the Bray-Curtis dissimilarity between initial litters and litters after 122 days of decomposition can explain 93% of the variation in mass loss under the moisture regime treatment, whereas only 30% was explained under the inundation regime. Together, we did not find marked differences in the responses of mass loss rate to N addition when litters were decomposed under the moisture regime treatment. This result indicates that inundation has greater impacts on litter residue chemistry than the conditions in the moisture regime.

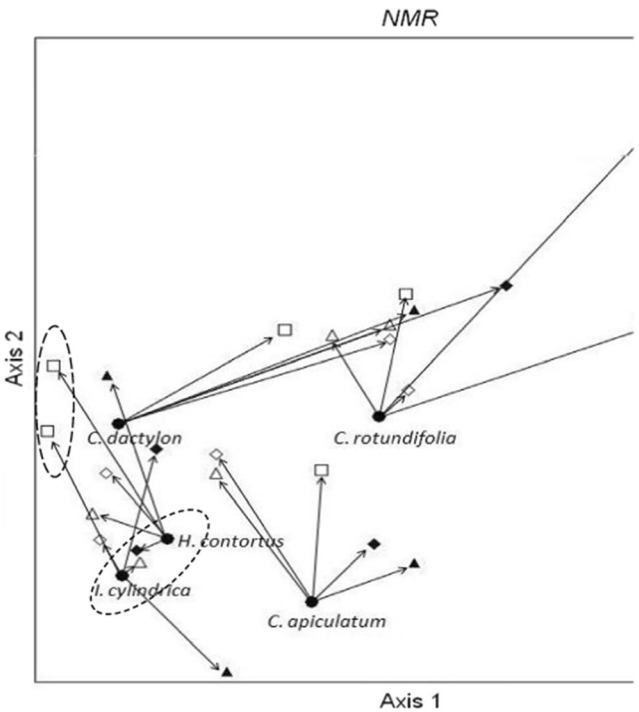

Fig. 3 Decomposition of different litters as represented by an NMDS plot using the litter NMR spectra parameters. Initial chemical variation of litters can be separated by axis 1. ● Initial of litters, ▵*S* inundation regime without N addition, ◇*S+N* inundation regime with N addition of 1 mg L^{-1}, □*S+3N* inundation regime with N addition of 3 mg L^{-1}, ▲*M* litter moisturized without N, and ◆ *M+N* moisturized with N addition

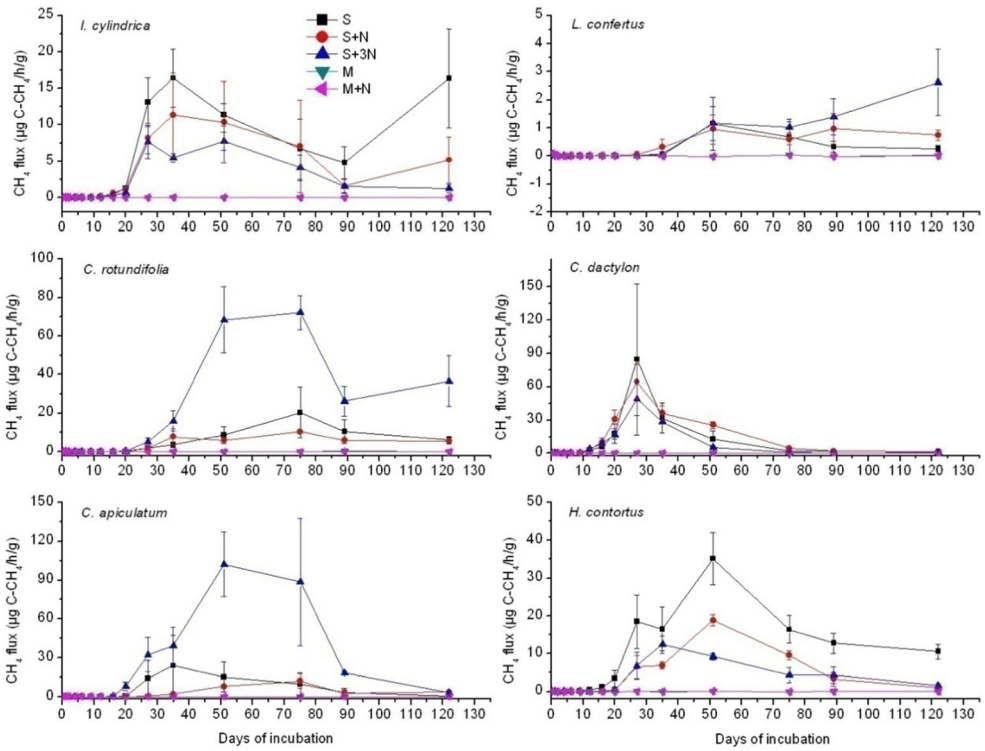

Fig. 4 CH_4 emissions from the litter microcosm. *Error bars* indicate SEs ($n = 3$). *S* inundation regime without N addition, *S+N* inundation regime with N addition of 1 mg L^{-1}, *S+3N* inundation regime with N addition of 3 mg L^{-1}, *M* litter moisturized without N and *M+N* moisturized with N addition

4.4 The response of CH_4 production to mass loss and litter chemical components

Although some studies have demonstrated the importance of anaerobic conditions for CH_4 production (Wang et al. 1993; Moore and Dalva 1997), methanogenic archaea can survive well in aerated environments and become active once under wet anoxic conditions (Angel et al. 2012). Decomposition of litters under flooded conditions in riparian areas does not occur in a strictly anaerobic environment. Thus, in the present study, the plant leaf litters were incubated under air conditions, which could allow the extrapolation of these results to the field scale. We observed marked emissions of CH_4 from flooded litter microcosms. This might be the case only if the bottom of the microcosms were anaerobic.

Notably, the difference in CH_4 emissions observed between litters was not significantly related with mass loss, and therefore did not support our first hypothesis. These results are consistent with Zak et al. (2015), in which no linear relationship between litter mass loss and CH_4 emission was found. In their study, they also used an incomplete anaerobic condition. In a mesocosm experiment using the plant tissues of five species from German fens, Zak et al. (2015) also found that *Ceratophyllum demersum* had the highest CH_4 production potential. In our study, the lower mass loss of *H. contortus* litters during decomposition produced more CH_4 compared to the rapid mass loss of *C. rotundifolia* and *C. dactylon* litters. Thus, the species-specific chemical quality of plant litters is likely to be an important factor leading to the uncertainty in CH_4 emission. These results suggest that mass loss is not an

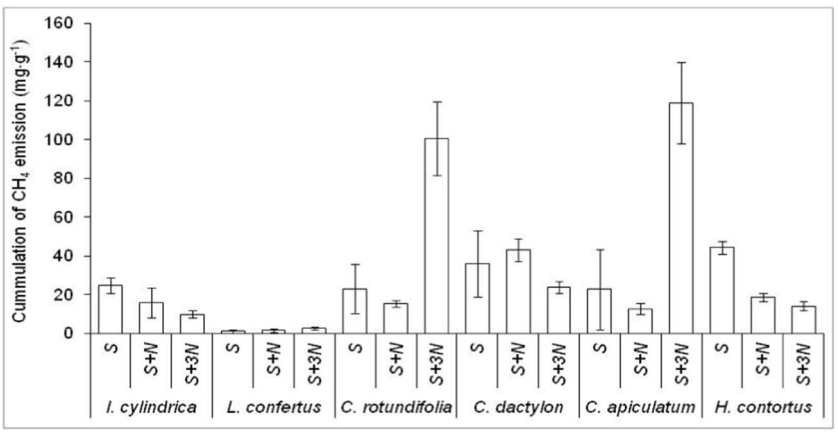

Fig. 5 Cumulative CH_4 emission during the incubation period. *S* inundation regime without N addition, *S+N* inundation regime with N addition of 1 mg L^{-1}, *S+3N* inundation regime with N addition of 3 mg L^{-1}, *M* litter moisturized without N and *M+N* moisturized with N addition

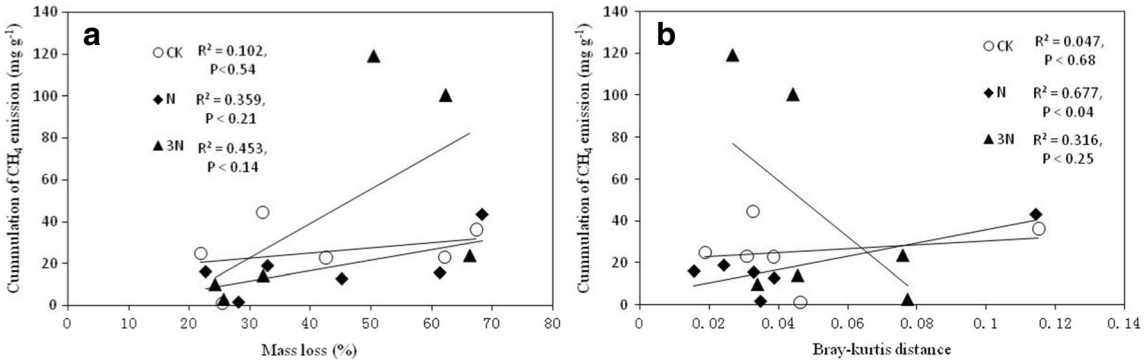

Fig. 6 Relationships between the Bray-Curtis distance of NMR chemical properties and **a** cumulative CH_4 emission and **b** mass loss of litters decomposed under the inundation treatment

ideal model parameter to predict CH_4 emission in an inundated field. The Bray-Curtis distance of NMR chemical properties between initial litters and litters that were decomposed 122 days cannot well explain the variation in CH_4 in inundation regime microcosms. Thus, our hypothesis that leaf litters that have undergone more alteration in chemical properties would stimulate increased CH_4 production was not supported.

Our observation indicating that N addition can either stimulate or inhibit CH_4 emission partially supported our third hypothesis. N fertilizers in most cases caused increases in CH_4 emissions from rice soils. In a microcosm experiment by Irvine et al. (2012), CH_4 emission potential was enhanced by N addition. In our study, the results from *C. rotundifolia* and *C. apiculatum* (class I) microcosms are consistent with most previous studies showing that N addition stimulated CH_4 emissions. In contrast, a decreased effect of N addition on CH_4 emission was observed for the litter of the three gramineous species, *I. cylindrica*, *C. dactylon* and *H. contortus* (class II), which shared a similar initial chemical composition. One interpretation is that such a difference in CH_4 production could be due to the variation in litter chemistry, showing an inhibitive effect on CH_4 net emissions during decomposition in the inundation regime. An alternative interpretation involves shifts in methanogenesis and methanotrophy in litter microcosms, as organisms in these two functional groups respond to N and C substrates differently. Because incubation was not strictly anaerobic, we cannot exclude the possibility that methanogenesis and methanotrophy co-occurred in the microcosms. If they are both stimulated and inhibited by N addition and other substrates, they may have masked equivalent increases in methane production and consumption. A final interpretation is that the introduction of nitrate may boost the growth of denitrifiers that can prohibit the methanogen activity. More work is needed to confirm these results in the litters of other plant species and using more N addition gradients to investigate whether these complicated responses of stimulation and inhibition are due to the variation in the chemical composition of litters, or in the abundance and/or composition of the methanotroph community or their metabolic efficiency.

In another field experiment, Vivanco et al. (2015) found that methane flux increased linearly with N addition at 7 months of fertilization but that there was no effect on CH_4 flux after 14 months of fertilization. Similarly, we also observed a significant temporal variation in the response of CH_4 to N addition in our microcosm study. An explanation for such a trend could occur as a result in the depletion of available C sources, causing declines in methanogen community abundance and activity.

Our results suggest that three stages can be identified during decomposition of inundated plant litters resulting in CH_4 net emissions, corresponding to the three-stage hypothesis of litter decomposition proposed by Berg and McClaugherty (2008). In the initial stage, the soluble components degrade rather quickly and hemicelluloses started to break down, fast-growing saprophytic bacteria dominate and no marked CH_4 emission can be detected at this stage. In the second stage, hemicelluloses and celluloses are predominantly degraded and small amounts of acid fermentation products such as acetic acid, lactic acid, propionic acid, and methanol, are produced and accumulated in the microcosms. These are necessary substrates for methanogenic archaea for the production of CH_4. Our results were similar to those of da Cunha-Santino and Bianchini (2013), showing the appearance of CH_4 from the 20th day of incubation. Some studies have shown that the emission of CH_4 is related to the hydrolysis of cellulose and enzyme activity of cellulose (Chin et al. 1999; Claassen et al. 1999; Wu and Conrad 2001). Thus, the variation in the time of appearance of rapid production of CH_4 among plant litters may be due to differences in cellulose content and the level of lignification of the holocellulose. In the final stage, the cellulose and hemicelluloses were mostly degraded, lignin dominated the residues and methanogen activity was suppressed.

4.5 Implications

Our results can provide some suggestions for reservoir dam management to reduce CH_4 emissions. Water level fluctuation and submerged plant materials in reservoir riparian zones commonly occur with heavy precipitation events and flood mitigation storage. In terms of mitigating CH_4 emission, the optimum would be to regulate water levels to prevent inundating riparian vegetation, especially *H. contorcus*. If inundation is inevitable, it may be feasible to shrink the duration of inundation so that it does not exceed 20 days. Another alternative approach to mitigate CH_4 emissions is to cut and harvest aboveground vegetation grass biomass before inundation due to impound. *H. contortus* is a native species of the tropics and subtropics of Australia, southern Asia and Africa and is widely grown in India, South America and the USA. The cellulose content of this grass species is quite high, and it is a palatable forage grass at a young age. However, *H. contortus* becomes undesirable and injurious to animals as it matures because its sharp-pointed seeds can penetrate livestock skin and mouth membranes. Thus, harvesting the aboveground biomass could be economically feasible before summer flooding to strongly reduce the high potential of CH_4 emissions. Although N addition can either stimulate or suppress CH_4 emissions, our study indicates that controlling the amount of N fertilizer used in upstream areas can regulate CH_4 emission in reservoir riparian ecosystems, considering the extent of stimulation or suppression on CH_4 emissions.

5 Conclusions

Decomposition of submerged plant litters was a vital biogeochemical process in seasonally flooded low-lying areas. Our results provide new evidence that mass loss and chemical composition of litters decomposed under flooded conditions differed markedly from the litters in the moisture regime treatment. We showed that labile leaf litters could fuel a rapid, high rate of methane production while recalcitrant litters could fuel lower emissions. We did not find that leaf litters that had undergone greater alteration in chemical properties stimulated more CH_4 production. Additionally, our results suggest that inundated litters are likely to be a marked source of CH_4 emission in areas with high reactive N input.

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