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Article The Impact of Modifications in Forest Litter Inputs on Soil N₂O Fluxes: A Meta-Analysis

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Abstract: Although litter can regulate the global climate by influencing soil N₂O fluxes, there is no consensus on the major drivers or their relative importance and how these impact at the global scale. In this paper, we conducted a meta-analysis of 21 global studies to quantify the impact of litter removal and litter doubling on soil N₂O fluxes from forests. Overall, our results showed that litter removal significantly reduced soil N₂O fluxes (-19.0%), while a doubling of the amount of litter significantly increased soil N₂O fluxes (30.3%), based on the results of a small number of studies. Litter removal decreased the N₂O fluxes from tropical forest and temperate forest. The warmer the climate, the greater the soil acidity, and the larger the soil C:N ratio, the greater the impact on N₂O fluxes associated with litter removal were greater in acid soils (pH < 6.5) or soils with a C:N > 15. Litter removal decreased soil N₂O fluxes from coniferous forests (-21.8%) and broad-leaved forests (-17.2%) but had no significant effect in mixed forests. Soil N₂O fluxes were significantly reduced in experiments where the duration of litter removal was <1 year. These results showed that modifications in ecosystem N₂O fluxes due to changes in the ground litter vary with forest type and need to be considered when evaluating current and future greenhouse gas budgets.

Keywords: litter; forest soil; greenhouse gases; N2O

1. Introduction

Since the 1970s, increasing attention has been directed at N₂O emissions, largely because they make a major contribution to the greenhouse gas (GHG) budget of managed ecosystems and have a large global warming potential [1]. In addition to its impact on global warming, N₂O is also the primary ozone-depleting substance in the stratosphere [2,3]. Although N₂O concentrations in the atmosphere are only one percent of the concentration of CO₂, N₂O has a global warming potential nearly 300 times that of CO₂ [4], and therefore, any changes in N₂O emissions could have a significant impact on the global climate even though the current contribution of N₂O to anthropogenic greenhouse gas emissions is lower (7%) than that of CO₂ (56%) [1].

Soil N₂O emissions are dependent on N availability and are affected by many environmental factors, such as soil stability [5], soil microbial communities [6], and plant litter inputs [7,8]. In particular, changes in above-ground litter inputs can have a major influence on soil biogeochemical processes by changing their physical and chemical properties



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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). (organic C and nutrient inputs) and/or by modifying the activities of soil microorganisms [9,10]. Forest soil is one of the major sources of N_2O in the atmosphere, and it has been estimated that N_2O is released from forest ecosystems to the atmosphere at a rate of 3.62 Tg N yr⁻¹ [11]. In 2020, forests covered 31% of the total land area, equivalent to about 4.06 billion hectares [12] and could make a potentially significant contribution to global N_2O budgets.

Climate change may affect litterfall in forests because of changes in rainfall patterns and increased temperatures. Increases in the average annual temperature can also affect tree phenology and tree species distribution [10,13], and increases in productivity and litterfall have been observed due to elevated atmospheric CO_2 concentration or temperature [14,15]. Whilst forest ecosystems are typically associated with significant litter accumulation, it is unclear how the litter layer affects soil N2O fluxes. Plant litter is an important source of organic matter, which can regulate soil physical and chemical properties, microbial biomass and activity, and affect soil N_2O emissions through the provision of readily available C, N, and other chemical components (for example, condensed tannins and terpenes) during the decomposition process [9,16]. Further, litter can modify soil temperature and water content by forming a barrier at the interface between the atmosphere and soil surface [17-19]. Despite the potentially important contribution of litter to N₂O emissions from forest ecosystems, there is currently no consensus on the direction or magnitude of any impact nor have the underlying drivers or their relative importance been resolved [20]. For example, some researchers have found that N_2O emissions decreased when litter was removed [21–25], while others have found that this had no significant influence on soil-atmosphere N₂O fluxes [9,26–28].

The inconsistencies in previous results may be due to several reasons, including differences in forest ecosystem type, characteristics, climatic conditions, litter volume, or experimental duration [29,30]. The results from a single study were often highly heterogeneous, making it difficult to integrate and analyze the effects of many of the above factors. To evaluate how litter changes affect soil N₂O fluxes of forests at a global scale, we extracted data on the effects of litter inputs on GHG fluxes from field experiments. We retrieved 21 published articles from a range of national and international studies to address the following two questions: (1) How do soil N₂O fluxes respond to litter changes in different forest types? (2) What key factors underpin the response of soil N₂O fluxes to litter changes?

2. Materials and Methods

2.1. Data Collection

We searched for peer-reviewed articles from the Web of Science, Google Scholar, and the China Knowledge Resource Integrated Database (CNKI) using the following research terms: litter removal/double litter/litter inputs, N₂O flux, and GHG flux. In order to specifically address the research questions identified and to reduce any bias brought about by screening the literature, we extracted papers that matched the following criteria: (a) The experimental studies were conducted in the field, and were not subjected to other confounding factors (e.g., fire, drought, irrigation, fertilization, and warming). (b) In the field experiments, the existing ground litter cover found under natural conditions was used as the control group, the litter removal treatment represented the complete removal of all aboveground litter, and the increased litter treatment involved a doubling of the existing ground litter cover. (c) The means, standard deviations/errors, and replication of variables in control and treatment groups could be extracted directly from the tables, or text, and/or by digitizing graphs. (d) When data from the same site and treatments were presented in multiple publications, we used the data from the most recent year.

Additionally, forest type (e.g., tropical forest and temperate forest), location (e.g., latitude and longitude), climatic information (e.g., MAT, mean average temperature and MAP, mean average precipitation), soil properties (e.g., soil pH and soil C:N), and experimental duration (year) were acquired directly from the papers. In total, 37 observations were extracted from 21 publications published during 2000–2020. By grouping MAT, MAP, HI (humidity index), soil pH, soil C:N, and experimental duration, we were able to explore the influence of climate conditions, soil properties, and experimental duration on soil N₂O fluxes. The sampling plot information is shown in Figure S1 in the Supplementary Material.

2.2. Meta-Analysis

The raw data were either obtained from tables or extracted by digitizing graphs using the GetData Graph Digitizer (version 2.24; http://getdata-graph-digitizer.com, accessed on 1 March 2022). If the data provided in the paper was the standard error (SE), the standard deviation (SD) could be converted using the formula shown below (1). In the three reports where SD or SE were not reported, SD was estimated as 10% of the mean [31,32]. The meta-analysis approach requires that all observations are independent. To meet these assumptions for the meta-analysis, where the temporal dynamics of a target variable was presented in the same studies, the average mean (\overline{M}) and the average standard error (\overline{SE}) of the variable were estimated using (2) and (3) [33]:

$$SD = SE\sqrt{N} \tag{1}$$

$$\overline{\mathbf{M}} = \sum_{i=1}^{j} \mathbf{M}_i / j \tag{2}$$

$$\overline{\mathrm{SE}} = \sqrt{\frac{\sum_{i=1}^{j} \mathrm{SE}_{i}^{2}(n_{i}-1)n_{i}}{\left(\sum_{i=1}^{j} n_{i}-1\right)\sum_{i=1}^{j} n_{i}}}$$
(3)

where *j* is the sampling times (≥ 2); M_{*i*}, SE_{*i*}, and n_{*i*} were mean value, standard error, and sample size on the *i*th sampling date, respectively.

The meta-analysis was carried out using the MetaWin software (Version 2.1; Sinauer Associates, Inc., Sunderland, MA, USA). The valid data for inputting into the model were the mean (Mean), standard deviation (SD), number of samples (n), and the categorical variables of N₂O fluxes in the treatment control groups. A response ratio (RR) was used to represent the degree of influence of litter alterations on N₂O fluxes. The RR value was calculated as the ratio of the mean value in the treatment group and that in the control group.

$$\ln RR = \frac{\ln X_e}{\ln X_c} = \ln X_e - \ln X_c \tag{4}$$

The corresponding variance (V) for each lnRR was calculated as follows:

$$V = \left(\frac{1}{n_{\rm e}}\right) \times \left(\frac{S_{\rm e}}{X_{\rm e}}\right)^2 + \left(\frac{1}{n_{\rm c}}\right) \times \left(\frac{S_{\rm c}}{X_{\rm c}}\right)^2 \tag{5}$$

where X_e and X_c are the means of the treatment and control groups, respectively; n_e and n_c are the corresponding sample numbers; and S_e and S_c are the corresponding standard deviations (SD).

The effect value of each pair of data was calculated, and then the combined effect value (RR⁺⁺) and 95% confidence interval (CI) were calculated using MetaWin 2.1. The specific related formulas are found in [34,35]. A positive value of RR⁺⁺ represented a positive effect, and a negative value of RR⁺⁺ represented a negative effect. The effect of litter change on N₂O fluxes within a categorical subdivision was considered to be significant at p < 0.05 if the 95% CI did not include 0. In addition, to facilitate the interpretation of the results, the percentage change in soil N₂O fluxes (%) was calculated using the following formula [36,37]:

$$Increase(\%) = \left(e^{RR++} - 1\right) \times 100\%$$
(6)

The figures in this paper were drawn in Origin (Version 9.0; OriginLab, Northampton, MA, USA) and correlations were tested using the SPSS software v19.0 for Windows (IBM, Chicago, IL, USA). Processing of all the original data was completed in Microsoft Excel v2016 (Microsoft, Washington, DC, USA).

3. Results

3.1. Effects of Litter Removal and Doubling on Soil N₂O Fluxes of Forests

Considering all forest types together, litter removal decreased soil N₂O fluxes by 19.1%, while litter doubling increased soil N₂O fluxes by 30.3% (Figure 1). Litter removal decreased the N₂O fluxes from tropical forest and temperate forest soils by 18.0% and 20.2%, respectively (Figure 1). Litter removal decreased soil N₂O fluxes from coniferous forests and broad-leaved forests by 21.8% and 17.2%, respectively. However, litter removal had no significant effect in mixed forests (Figure 1).



Figure 1. Effects of litter removal or a doubling of litter inputs on soil N₂O fluxes for different forest ecosystem types. Horizontal lines represent the 95% confidence intervals (CI) and the numbers of observations are included in parentheses. The means were considered significantly different if their 95% CI did not overlap with zero. TRF, tropical forest; TF, temperate forest; BLF, broad-leaved forest; MF, mixed forest; CF, coniferous forest.

3.2. Influence of Climate Factors on Soil N₂O Fluxes in Response to Litter Manipulations

The response of soil N₂O fluxes to litter removal was also affected by climatic factors and only significantly reduced in regions where the MAT was >20 °C or where the MAP was between 500 and 1000 mm (Figure 2a,b). The soil N₂O flux was significantly reduced after litter removal in humid regions with an HI value >60 (22.6%) and medium humid regions with an HI between 30 and 60 (12.6%) (Figure 2c). Due to insufficient data from in situ experiments, it was not possible to evaluate the effects of climate factors on the response of N₂O fluxes to litter doubling.



Figure 2. Effects of litter removal and a doubling of litter inputs on soil N_2O fluxes for different values of MAT ((**a**), mean average temperature), MAP ((**b**), mean average precipitation), HI ((**c**), humidity index), soil pH (**d**), soil C: N ratio (**e**), experimental duration (**f**). Horizontal lines represent the 95% confidence intervals (CI) and the number of observations is included in parentheses. The means were considered significantly different if their 95% CI did not overlap with zero.

3.3. Influence of Other Factors on Soil N₂O Fluxes in Response to Litter Manipulations

The decrease in soil N₂O fluxes associated with litter removal was greater in acid soils (pH < 6.5) (Figure 2d). The response of soil N₂O fluxes to litter removal was also affected by the soil C:N ratio and significantly reduced in regions where the soil C:N was >15 (Figure 2e). The experimental duration also influenced the effect of litter removal on soil N₂O fluxes, and only decreased significantly when the experimental duration was less than 1 year (Figure 2f). Again, due to insufficient data from in situ experiments, it was not possible to evaluate the effects of soil pH, soil C:N ratio, and experimental duration on the response of N₂O fluxes to litter doubling.

4. Discussion

4.1. The Effect of Litter Removal and Doubling on Soil N₂O Fluxes

Litter plays an important impact in modifying nutrient retention and availability in forest ecosystems, and litter decomposition is an important route through which nutrients present in the vegetation are returned to the soil [38–40]. Therefore, changes in litter inputs may affect soil-atmosphere N₂O fluxes by changing the availability of labile organic substrates and inorganic nutrients [41]. While, overall, our results showed that litter removal significantly reduced soil N₂O fluxes from forests, there is a paucity of data from litter doubling treatments, although the limited available data does suggest that litter doubling can increase N₂O fluxes from forest soils. Litter can influence N transformations in the soil that lead to N_2O emissions through the addition of both C and N substrates as well as providing nitrate as an alternative electron acceptor for denitrification processes under low oxygen conditions [42,43]. In addition, an anaerobic environment can be formed due to the consumption of soil O_2 in soil respiration, which may indirectly enhance denitrification, thereby increasing N_2O emissions [44,45]. However, the contrasting impacts with different forest types indicate that other factors are involved, and litter removal will not always lead to a decrease in N_2O emissions, with the overall impact globally dependent on the relative proportion of each forest type.

The inputs of exogenous organic matter often change the diversity and number of microorganisms in the soil, and increases in the contribution of root turnover and root exudates for supporting microbial growth and metabolism could potentially stimulate the decomposition of organic nitrogen in the soil [46,47]. Therefore, we speculate that N_2O emissions are more sensitive to litter doubling than litter removal. In our study, we found differences in the effect of litter doubling and removal on soil N₂O fluxes from forests in 21 studies, and litter doubling had a greater effect (30.3%) on N_2O emissions than litter removal (19.1%). However, we only focused on three studies that included both litter removal and doubling to conduct an integrated analysis, and in this case, inconsistent results were found and the effects of litter removal (31.8%) on N₂O emissions were only slightly greater than that of litter doubling (30.1%). Clearly, any definitive conclusions are limited by the paucity of available data, and we cannot infer the effect of litter addition on soil N_2O emissions based on the effects of litter removal (opposite sign), due to differences in the effects of the two treatments. Considering the likely increase in plant productivity brought about by future global climate change, this will lead to an increase in litter production and a likely increase in the magnitude of the impact on soil N₂O fluxes, a factor that should be considered when modeling soil N₂O emissions from terrestrial ecosystems. Therefore, further attention needs to be paid to the impact of litter addition on soil N_2O emissions as one of the effects of climate change on forest ecosystems.

4.2. Factors Affecting the Response of Soil N₂O Fluxes to Changes in Litter Inputs

Tropical forest (especially tropical rainforests) soils are considered to be the main source of atmospheric N₂O due to the improved hydrothermal conditions (higher temperatures and higher water availability) and a higher soil microbial activity [48,49]. Globally, the total N₂O efflux from tropical rain forests has been reported to be 1.34 Tg N yr⁻¹, which accounts for about 18% of the total N₂O efflux from soil under natural vegetation [50]. Considering the seasonality of litter inputs, vegetation degradation, and climate condition, the production and decomposition of litter in tropical forests are larger and faster than in temperate forests [51]. Therefore, we assumed that the impact of litter removal on soil N₂O fluxes in tropical forests was greater than that in temperate forests, but our results are inconsistent with this. We found litter removal reduced soil N₂O fluxes in tropical and temperate forests. The higher temperature conditions, water availability, and the greater substrate availability in tropical forests may contribute to form a thicker humic layer [52,53]. The recalcitrant humic materials in an advanced state of decay contribute to relatively high soil nitrogen contents in tropical forests [54], which may alleviate the impact of litter removal on soil N₂O.

Forest type also influenced the response of N_2O fluxes to litter inputs. Chen [55] found that the impact of litter removal on broad-leaved tree species was greater than on coniferous tree species (coniferous forest) in field experiments, which was inconsistent with the global research results of this study. Our results showed that litter removal decreased soil N_2O fluxes from coniferous forests and broad-leaved forests, especially in coniferous forests. However, litter removal had no significant effect in mixed forests. This may be due in part to differences in nutrient availability. Du et al. [56] predicted global patterns of N and P availability, and overlapped their predictions with a global map of major terrestrial biomes, and found that N limitation was relatively more common in boreal and temperate coniferous forests, whereas P was more restricted in tropical and subtropical forests, temperate broadleaf, and mixed forests. Under N limited conditions, litter removal may significantly reduce the N_2O fluxes indicating that soil N availability has a key role to play in determining the effect of changes in external litter inputs [25].

The response of soil N_2O emissions to litter inputs was affected by soil pH. The N_2O flux of acid soils (<6.5) decreased significantly after litter removal. As acid rain is often associated with acid soils, this may be because litter can modify soil pH changes consistent with the results of Zhong et al. [57] who found that soil pH increased with litter inputs. Litter also provides cations to the soil during the decomposition process, which can slow down soil acidification. Litter removal leads to increased acidification of the soil, decreasing the number of nitrifying bacteria, and inhibiting nitrification which, in turn, significantly reduces N_2O emissions [58]. At the same time, the high concentration of hydrogen ions in acidified soil disperses soil aggregates into single particles and breaks down the soil structure [59], indirectly affecting the abundance of soil nitrifying and denitrifying bacteria. The presence of litter can also reduce the leaching of soil N and P and their impact on N_2O emissions [59]. The effect of litter removal on N_2O emissions was also affected by the soil C:N ratio and significantly reduced in regions where the soil C:N was >15. A high soil C:N ratio promotes an increase in soil microbial populations and greater response of soil N₂O fluxes to litter inputs [36,60]. Short-term (<1 year) litter removal experiments were associated with significantly reduced soil N2O emissions, while longer-term litter removal had no significant effect on the emissions that could be related to a time-dependent effect. As well as the above factors, litter quality and quantity can also have a significant impact on the response of N₂O emissions to litter inputs. However, due to insufficient information, a more detailed assessment of the influence of litter C:N ratio on soil N₂O emissions awaits further experimental studies.

5. Conclusions

Our study found that litter removal significantly reduced N_2O emissions from forest soils, while a few existing studies found that doubling the amount of litter significantly increased emissions. Underlying this were differences among the forest types examined, which were related to climatic conditions, soil pH, soil C:N, and experimental duration. Litter removal significantly reduced soil N₂O fluxes in tropical and temperate forests. The decrease in soil N_2O fluxes associated with litter removal was greater in acid soils (pH < 6.5) or soil C:N > 15. Litter removal had a greater effect on N_2O fluxes from coniferous forest, which may be due to the low availability of N, emphasizing the importance of an improved understanding of how interactions with soil properties determine litter-related emissions. Experimental duration was also found to have an important impact on N_2O emissions; short-term (<1 year) litter removal experiments were associated with significantly reduced soil N₂O emissions. It was often considered reasonable in many meta-analyses to estimate the SD as 10% of the mean when standard errors were not available, this may have led to errors in some biological and empirical systems. However, the potential errors are likely to be small because there were only a few values (three values) where we had to do this. Overall, these results show that changes in the ground litter of forests have the potential to modify ecosystem N₂O fluxes and need to be considered when evaluating current and future GHG budgets. Considering the likely increase in plant productivity brought

about by future global climate change, this should lead to an increase in litter production. Therefore, further studies are required to investigate the impact of litter addition on soil N_2O emissions in the future in order to fully assess the effect of climate change on forest ecosystems. In addition, the majority of litter production studies have been conducted in temperate and tropical forests, whilst studies in other forest systems, especially boreal forests, have received less attention.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/atmos13050742/s1, Figure S1: Global distribution of sampling sites used for this meta-analysis; Table S1: The relationships between the response ratios (RR) and its relevant factors.

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