

# Different responses of terrestrial soil microbial biomass to the addition of varied types of nitrogen fertilizers

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## Abstract

Atmospheric deposition of nitrogen (N) compounds have significantly increased worldwide in the recent decades, mainly in the form of ammonium ( $\text{NH}_4^+\text{-N}$ ), nitrate ( $\text{NO}_3^-\text{-N}$ ) and urea ( $\text{CO}(\text{NH}_2)_2$ ). Here, we used meta-analysis to compare the influence of different N types on terrestrial soil microbial biomass based on 1585 paired observations from 178 articles. In all N types,  $\text{NH}_4^+\text{-N}$  addition displayed the greatest negative affects on soil microbial biomass (-18.9 %) followed by.  $\text{NH}_4\text{NO}_3$  (-7.51 %) and  $\text{NO}_3^-\text{-N}$  (-7.26 %).  $\text{CO}(\text{NH}_2)_2$  addition resulted in the smallest total microbial biomass declines among all N types (-6.99 %). All the soil microbial characteristics, such as fungi and bacteria, revealed the same trends across all ecosystems, especially for arbuscular mycorrhizal fungi, F/B, and G+/G- ratio. In addition, the response sizes were significantly correlated with the accumulated N amount (N addition rate  $\times$  application duration). The results indicated that the response sizes were significantly N-type dependent.

## INTRODUCTION

Atmospheric nitrogen (N) deposition has increased markedly since the middle of the 20<sup>th</sup> century. The enhanced N has been acknowledged as a global and increasing threat to biomass, biodiversity (Sala *et al.* 2000) and ecosystem function (Phoenix *et al.* 2012). For plants, N is a driving force of plant diversity (Zong *et al.* 2019) and ecosystem functioning (Dias *et al.* 2014). For soil microbial community, N deposition may increase (Liu & Zhang 2019) or decrease soil microbial biomass (Zhou *et al.* 2019) and diversity (Wang *et al.* 2018a). Many short- and long-term projects have been executed to investigate the effects of increased N on soil microorganisms in terrestrial ecosystem, such as the Dinghushan Biosphere Reserve (DHSBR) Experiment (Wang *et al.* 2018b), Changbaishan Forest Ecosystem Research Station (CBFERS), and Inner Mongolian Grassland Experiment (Li *et al.* 2017) in China, Harvard Forest Experiment in the USA (Turlapati *et al.* 2013), and Nitrogen Saturation Experiment (NITREX) in Europe (Moldan *et al.* 2018). Accumulative N fertilization has been shown to cause positive (Tahovská *et al.* 2020) or negative (Zhang *et al.* 2018) effects on soil microbial biomass. In their reports, however, ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ) was usually selected as the N fertilizer (Chen *et al.* 2018; Liu & Zhang 2019; Luan *et al.* 2019), followed by urea ( $\text{CO}(\text{NH}_2)_2$ ) (Li *et al.* 2017), while ammonium ( $\text{NH}_4^+\text{-N}$ , e.g.,  $\text{NH}_4\text{Cl}$ ,  $(\text{NH}_4)_2\text{SO}_4$ , and  $(\text{NH}_4)_3\text{PO}_4$ ) and nitrate ( $\text{NO}_3^-\text{-N}$ , e.g.,  $\text{NaNO}_3$ ,  $\text{KNO}_3$ , and  $\text{Ca}(\text{NO}_3)_2$ ) were less used (Liu *et al.* 2016; Zhang *et al.* 2017). Actually, the anthropogenic N from atmosphere is a set of mixture, mainly in the form of  $\text{CO}(\text{NH}_2)_2$ ,  $\text{NH}_4^+\text{-N}$ , and  $\text{NO}_3^-\text{-N}$  (Cornell 2011).  $\text{CO}(\text{NH}_2)_2$  is usually used as organic N fertilizer in agricultural activities. The  $\text{NH}_4^+\text{-N}$  is closely associated with intensive livestock husbandry and ammonia ( $\text{NH}_3$ ) emission (Zhou *et al.* 2019). The source of  $\text{NO}_3^-\text{-N}$  is nitrogen oxide ( $\text{NO}_x$ ), which are closely associated with industrial activities, such as fossil fuel combustion (Liu *et al.* 2013).

Some studies have shown that the addition of different N types induced different soil and microbial characteristics changes. For soil characteristics, the application of  $\text{NO}_3^-\text{-N}$  fertilization did not significantly affect soil pH, but  $\text{NH}_4^+\text{-N}$  application significantly decreased its pH (Paredes *et al.* 2011).  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  also

revealed different effects on soil organic matter decomposition (Yang *et al.* 2014; Khalil *et al.* 2016). For microbial biomass,  $\text{CO}(\text{NH}_2)_2$  addition significantly increased soil microbial biomass (+12.1%), while  $\text{NH}_4\text{NO}_3$  did not significantly affect soil microbial biomass (Treseder 2008). For enzymatic activities,  $\text{NH}_4\text{NO}_3$  deposition inhibited microbial enzymatic activities, but  $\text{CO}(\text{NH}_2)_2$  deposition promoted the processes (Du *et al.* 2014).  $N$ -acetyl-glucosaminidase, cellobiohydrolase, phosphatase, and phenol oxidase activities increased after  $\text{NH}_4^+$ -N addition. By contrast,  $\text{NO}_3^-$ -N fertilization did not significantly affect the activities of  $\alpha$ -glucosidase, cellobiohydrolase,  $\beta$ -xylosidase, and phosphatase (Currey *et al.* 2010). Therefore, it is essential to systematically compare the effects of different N fertilizers on ecosystem, especially under the same dose.

The responses of soil microbial biomass to N addition may be affected by the ecological factors of experiment site as well. For example, soil microbial biomass to N addition at high altitude and latitude regions was different from other regions (Fu & Shen 2017). N addition rates and latitude might directly and negatively affect the effects of N addition on N resorption efficiency (You *et al.* 2018). The responses of soil microorganisms to N addition were also affected by experimental duration, precipitation and soil type (Jia *et al.* 2020). Thus, it is also important to compare the effects of various N types on soil microorganisms under different ecological factors.

In terrestrial ecosystems, soil microorganisms are critical drivers of plant diversity and play important roles in ecosystem function (Liet *et al.* 2019). Here, we focused on soil microbial biomass and analyzed the impact of different types of N on them with meta-analysis. Based on previous findings, we hypothesized that: (1) soil microbial biomass would increase greater after  $\text{CO}(\text{NH}_2)_2$  addition compare with other N types. (2) Soil microbial biomass would increase significantly after  $\text{NH}_4^+$ -N addition as  $\text{NH}_4^+$ -N is thought to be the essential component for amino acid. (3) The effect of  $\text{NO}_3^-$ -N on soil microbial biomass would be less significant as  $\text{NO}_3^-$ -N is more mobile and subjected to leaching, especially in the area with greater precipitation. (4) The effects of  $\text{NH}_4\text{NO}_3$  addition on soil microbial biomass would be less than  $\text{NH}_4^+$ -N but greater than  $\text{NO}_3^-$ -N. (5) Soil microbial biomass with lower background N deposition level might be more sensitive to N addition.

## MATERIALS AND METHODS

### Data collection

Data were collected from peer-reviewed journal articles. These articles were searched by ISI Web of Science, ScienceDirect, Springer, Wiley, and Google Scholar. The search terms were “(nitrogen addition OR nitrogen deposition OR nitrogen fertilization) AND (microbial biomass OR fungal biomass OR bacterial biomass).” If these papers were selected for further analysis, they must meet the following criteria: (1) Only original research papers were included. (2) Experiments were conducted in terrestrial ecosystems. Laboratory incubation and agro-system experiment were excluded, as it is hard to estimate the background N deposition level and ecological factors. (3) Data only related to control and N addition treatment were extracted. Data with treatments of warming,  $\text{CO}_2$ , water or phosphorous addition plus N addition were excluded. (4) Means, sample sizes, and standard deviations or standard errors of soil microbial characteristics of both control and N fertilized treatments were extracted. (5) For N types,  $\text{NH}_4\text{Cl}$ ,  $(\text{NH}_4)_2\text{SO}_4$  and  $(\text{NH}_4)_3\text{PO}_4$  were classified as  $\text{NH}_4^+$ -N, while  $\text{NaNO}_3$ ,  $\text{KNO}_3$  and  $\text{Ca}(\text{NO}_3)_2$  were classified as  $\text{NO}_3^-$ -N. N fertilization rate was measured as N per unit area per year ( $\text{kg N ha}^{-1}\text{yr}^{-1}$ ).

Soil microbial characteristics, such as total microbial biomass, fungal biomass, bacterial biomass, actinomycete biomass, saprophytic fungal biomass, arbuscular mycorrhizal (AM) fungal biomass, gram-positive ( $G^+$ ) bacterial biomass, gram-negative ( $G^-$ ) bacterial biomass, as well as fungi to bacteria (F/B) ratio and  $G^+$  bacteria to  $G^-$  bacteria ( $G^+/G^-$ ) ratio, were extracted. Data of field experiment such as N application rate ( $\text{kg N ha}^{-1}\text{yr}^{-1}$ ) and duration (yr) were also extracted. Information of sample site, such as ecosystem types, background N deposition level ( $\text{kg N ha}^{-1}\text{yr}^{-1}$ ), mean annual temperature (MAT,  $^\circ\text{C}$ ) and mean annual precipitation (MAP,  $\text{mm yr}^{-1}$ ) were separately recorded as well. If the data of background N deposition of sample sites were missing, we used the “Nitrogen deposition onto the United States and Western Europe” dataset to estimate the background level (Holland *et al.* 2005) and extracted the information

from <http://nadp.slh.wisc.edu/>. If the sample site was located in China, we extracted the information from “National Science & Technology Infrastructure” from <http://www.cnern.org.cn>. If the MAT and MAP were not reported in the papers, we extracted the information from the database at <http://www.worldclim.org/> by latitude and longitude. Finally, our database included 1585 paired observations (**Supplementary Online Material 1**) from 178 articles (**Supplementary Online Material 2**) were classified into six ecotypes: desert, forest (including broadleaved and coniferous forest), grassland, tundra, shrub, and wetland around the world (**Supplementary Online sMaterial 3**).

## Statistical analysis

For each study, meta-analysis requires the mean, standard deviations (SD) and replicate number ( $n$ ) for the N fertilized ( $X_F$ ) and control ( $X_C$ ) treatments. All these data were extracted from the text, tables, figures, or supplemental materials of these publications. If data were presented by figures, Engauge Digitizer ( $v$  10.4) was used to obtain numeric data (<https://github.com/markummitchell/engauge-digitizer/releases>). If standard errors (SE) were reported, these were transformed to standard deviation (SD) with to the equation:  $SD = SE \times \sqrt{n}$ . Unidentified error bars were assumed to represent SE.

Natural log response ratio ( $\ln RR$ ) was calculated to access the responses of soil microbial biomass to N fertilization (Hedges *et al.* 1999).  $\ln RR$  was calculated as:

$$\ln RR = \ln(X_F/X_C) \quad (1)$$

The variance ( $v_i$ ) of  $\ln RR$  was approximated as:

$$v_i = \frac{(SD_F)^2}{n_F(X_F)^2} + \frac{(SD_C)^2}{n_C(X_C)^2} \quad (2)$$

where  $SD_F$  and  $SD_C$  are the SD for the fertilized and control treatments, respectively;  $n_F$  and  $n_C$  are the sample sizes in fertilized and control treatments, respectively

In the analysis, we used the number of replications for weighting:

$$Wr = (N_F \times N_C)/(N_F + N_C) \quad (3)$$

where  $Wr$  is the weight associated with each  $\ln RR$  observation,  $N_F$  and  $N_C$  are the numbers of replications in the fertilized and control.

Publication bias was assessed using funnel plots, by applying a regression test for funnel plot asymmetry (Veroniki *et al.* 2016). Normal quantile–quantile (Q-Q) plots were inspected to assess normality of the residuals. Only the results where corresponding Q-Q and funnel plots were satisfactory are presented here. For each mean effect size, 95 % confidence interval (CI) were calculated. If the 95 % CI of  $\ln RR$  did not overlap with 0, the effect of N fertilization on the variable differs significantly at  $\alpha = 0.05$  between the N fertilized and control treated. The percentage changes were presented in the figures as back transformed from the log response ratio ( $[e^{\ln RR} - 1] \times 100\%$ ). Analysis of variance (ANOVA) was used to compare the effects of different N on soil microbial biomass.

In order to estimate whether the responses of soil microbial biomass ( $\ln RR$ ) was significantly affected by ecological factors (such as MAT, MAP, background N deposition level, N application rate and application duration) under different N types, regression analyses were used (Humbert *et al.* 2016; Zhang *et al.* 2018). The restricted maximum likelihood (REML) method was used to produce final models, which were checked to ensure they conformed to modelling assumptions. The analyses were conducted using the *lme4* package in R version. The model were established as follows:

$$\eta = A_0 + A_1 \times F_1 + A_2 \times F_2 + A_3 \times F_3 \cdots A_i \times F_i \quad (4)$$

In this model,  $\eta$  is the response ratio ( $\ln RR$ ) of various microbial characteristics, such as fungal biomass;  $F_1, F_2 \cdots F_i$  are variates that may affect  $\ln RR$ , such as N addition rate, N fertilized duration, MAT and MAP;  $A_0, A_1, A_2 \cdots A_i$  are coefficients of each variates. In some models, some variates were transformed. **Supplementary Online Material 4** listed all the 13 assumed models. Akaike information criterion

(AIC) values were used to compare the fit of each model to the data. The model with lower AIC value was the best-fit model.

All the statistical analyses were performed using *R* 3.5.3 (*R* Core Team, 2018). The codes were shown in **Supplementary Online Material 5**.

## RESULTS

Across all the studies, the mean effect size of N addition on soil total microbial biomass was -7.69 % ( $P < 0.01$ ). However, the responses of total microbial biomass to various N types were different.  $\text{NH}_4^+$ -N addition led to a rather significant microbial biomass decline than other N types (-18.9 %,  $P < 0.01$ ). In all types of  $\text{NH}_4^+$ -N fertilizers,  $(\text{NH}_4)_2\text{SO}_4$  induced the most aggravated microbial biomass decline (-21.3 %,  $P < 0.01$ ).  $\text{NO}_3^-$ -N addition also significantly inhibited total microbial biomass (-7.26 %,  $P < 0.05$ ) but significantly less than  $\text{NH}_4^+$ -N (ANOVA,  $P < 0.05$ ). In all types of  $\text{NO}_3^-$ -N fertilizers,  $\text{KNO}_3$  addition induced the most serious microbial biomass decline (-21.8 %,  $P < 0.01$ ). Contrarily, total microbial biomass increased when  $\text{Ca}(\text{NO}_3)_2$  was fertilized (+3.66 %).  $\text{NH}_4\text{NO}_3$  addition significantly decreased microbial biomass as well (-7.51 %,  $P < 0.01$ ), but also less than single  $\text{NH}_4^+$ -N (ANOVA,  $P < 0.05$ ).  $\text{CO}(\text{NH}_2)_2$  addition induced the smallest microbial biomass decrease (-6.99 %,  $P < 0.05$ ) among all N types (**Fig. 1**).

Further analysis revealed that different soil microbial characteristics displayed various responses to these N fertilizers. Soil fungal biomass significantly decreased after N addition (-14.2 %,  $P < 0.01$ ). Among all types of N fertilizers,  $\text{NH}_4^+$ -N induced to the greatest fungal biomass (-47.6 %,  $P < 0.01$ ) than other N types (ANOVA,  $P < 0.05$ ), especially for  $(\text{NH}_4)_2\text{SO}_4$  (-54.5 %,  $P < 0.01$ , **Fig. 2A**, **Supplementary Online Material 6**). Similarly, soil bacterial biomass significantly decreased after N addition (-11.3 %,  $P < 0.05$ ). For all types of N fertilizers, bacterial biomass decreased the most after  $\text{NH}_4^+$ -N addition (-30.2 %,  $P < 0.01$ ) than others (ANOVA,  $P < 0.05$ ), while  $\text{CO}(\text{NH}_2)_2$  addition did not significantly affect them ( $P = 0.071$ , **Fig. 2B**, **Supplementary Online Material 6**). Because of the less decreases of bacterial biomass than fungal biomass, F/B ratio significantly declined for after N addition (-7.70 %,  $P < 0.01$ ). F/B ratio declined the most after  $\text{NH}_4^+$ -N addition (-27.9 %,  $P < 0.01$ ).  $\text{CO}(\text{NH}_2)_2$  addition also induced to significant F/B ratio decline (-15.6 %,  $P < 0.05$ ), but less than  $\text{NH}_4^+$ -N (ANOVA,  $P < 0.05$ ). However, F/B ratio did not significantly change by  $\text{NH}_4\text{NO}_3$  addition ( $P = 0.4288$ , **Fig. 2C**, **Supplementary Online Material 6**).

The biomass of both actinomycete and saprophytic fungi did not significantly change after N addition ( $P = 0.403$  and  $0.973$ ). The changes were also not significantly different among various N types, although actinomycete biomass significantly increased after  $\text{NH}_4^+$ -N addition (-26.3 %,  $P < 0.05$ , **Fig. 2D, E**, **Supplementary Online Material 6**). AM fungal biomass decreased the most (-27.1 %,  $P < 0.01$ ) among all microbial characteristics. Furthermore,  $\text{NH}_4^+$ -N addition induced to the most serious decline (-37.7 %,  $P < 0.05$ ), while  $\text{CO}(\text{NH}_2)_2$  led to more significantly biomass decline compared with  $\text{NH}_4\text{NO}_3$  (-36.9 vs. -20.9 %, ANOVA,  $P < 0.05$ , **Fig. 2F**, **Supplementary Online Material 6**). Both  $G^+$  and  $G^-$  bacterial biomass did not statistically significant change after N addition ( $P = 0.8088$  and  $0.2235$ , **Fig. 2G, H**, **Supplementary Online Material 6**). However,  $G^+/G^-$  ratio showed significant increase after  $\text{CO}(\text{NH}_2)_2$  addition (+14.4 %,  $P < 0.01$ ), but was insignificant affected by  $\text{NH}_4\text{NO}_3$  (**Fig. 2I**, **Supplementary Online Material 6**).

There were significant differences in soil microbial characteristics changes among ecosystem types. In general, N addition induced significant total microbial biomass decrease in forest tundra (-11.5 %,  $P < 0.01$ ), grassland (-10.9 %,  $P < 0.01$ ), forest (-6.90 %,  $P < 0.01$ ), and shrub (-4.77 %,  $P < 0.05$ ), but increase in wetland (+12.9 %,  $P < 0.05$ ). In all types of ecosystems, microbial characteristics decreased the most after  $\text{NH}_4^+$ -N addition. Compared with broadleaved forest (-2.70 %), in forest ecosystem, soil microbial characteristics in coniferous forest decreased more seriously (-3.51 %) after N addition for all N types, especially after  $\text{NH}_4\text{NO}_3$  addition (-2.70 vs. -7.44 %, ANOVA,  $P < 0.05$ , **Table 1**, **Supplementary Online Material 7**).

The results of the regression analyses showed that soil microbial responses to N addition were significantly correlated with the N addition rate and application duration (**Supplementary Online Material 4**). Among all the types of N fertilizers,  $\text{NH}_4^+$ -N addition revealed the most serious total soil microbial biomass

decline and  $\text{CO}(\text{NH}_2)_2$  addition induced to the smallest decline (**Fig. 3**). Various microbial characteristics revealed similar trends (**Supplementary Online Material 4**).

## DISCUSSION

In general, we found significantly negative effects of N addition on total microbial biomass. Further analysis revealed that total microbial biomass displayed different responses to various N types. As an important component of amino acids,  $\text{NH}_4^+\text{-N}$  is the main N source for soil microorganisms (Guo *et al.* 2011; Du *et al.* 2014). Its addition may significantly accelerate the metabolism of soil microorganisms. In contrast to our hypothesis,  $\text{NH}_4^+\text{-N}$  addition led to a rather significant microbial biomass decline than other N types across all ecosystems. This might be because the absorbed  $\text{NH}_4^+\text{-N}$  would be replaced by  $\text{H}^+$  in soils, which significantly decrease soil pH (Hong *et al.* 2019) and eventually induces serious soil microbial biomass decline. Of all N types,  $\text{NH}_4^+\text{-N}$  addition induced the greatest soil pH decline and displayed the most negative effect on microbial biomass especially for  $(\text{NH}_4)_2\text{SO}_4$  (-21.3 %,  $P < 0.01$ , **Supplementary Online Material 8**). In addition,  $\text{NH}_4^+\text{-N}$  additions may aggravate nitrification in soils. The nitrification process may be toxic to microorganisms, and then lead to a decrease in the microbial biomass (Zhang *et al.* 2017).  $\text{NO}_3^-\text{-N}$  addition also significantly inhibited total microbial biomass but less than  $\text{NH}_4^+\text{-N}$ . Firstly, the excessive addition of  $\text{NO}_3^-\text{-N}$  increased the activities of nitrate reductase and the accumulation of nitrite, which might be harmful to soil microorganisms (Luan *et al.* 2019). Secondly, as a type of anion,  $\text{NO}_3^-\text{-N}$  is more mobile and subjected to leaching (Currey *et al.* 2010). The direct negative effect of  $\text{NO}_3^-\text{-N}$  on microorganisms may be less obvious than  $\text{NH}_4^+\text{-N}$ . The significantly negative relationship between microbial biomass decrease and MAP supports our hypothesis (**Supplementary Online Material 10**). Thirdly, the ability of soil microorganisms to absorb  $\text{NO}_3^-\text{-N}$  may be lower (Fu & Shen 2017). Most soil microorganisms cannot immobilize  $\text{NO}_3^-\text{-N}$  under N addition (Brenner *et al.* 2005). Finally, it induced less soil acidification (**Supplementary Online Material 8**), which may reduce the negative effect of  $\text{NO}_3^-\text{-N}$  on soil microorganisms.

As the most used fertilizer in field experiment,  $\text{NH}_4\text{NO}_3$  addition significantly decreased microbial biomass as well, but less than single  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ . The reason may be its less acidification than single  $\text{NH}_4^+\text{-N}$  and less nitrite accumulation than single  $\text{NO}_3^-\text{-N}$  under the same dose. Based on the results, we hypothesized that N addition with extremely massive  $\text{NH}_4^+\text{-N}$  or  $\text{NO}_3^-\text{-N}$  may destroy the original balance of  $\text{NH}_4^+\text{-N}$  to  $\text{NO}_3^-\text{-N}$  in microorganisms and finally cause potential toxic effects on them. If  $\text{NH}_4\text{NO}_3$  was fertilized in the area with extremely higher or lower background level of  $\text{NH}_4^+\text{-N}/\text{NO}_3^-\text{-N}$  ratio, the results might mislead the estimation of the effect of elevated natural N deposition on the ecosystem.  $\text{CO}(\text{NH}_2)_2$  is a type of organic N and commonly used as fertilizers in agro-ecosystems. It was also used in some field experiments (Li *et al.* 2017; Zong *et al.* 2019). In this work,  $\text{CO}(\text{NH}_2)_2$  addition induced the smallest microbial biomass decrease. Microbial biomass even accelerated after  $\text{CO}(\text{NH}_2)_2$  addition in some reports (Thirukkumaran & Parkinson 2000; Guo *et al.* 2011; Du *et al.* 2014). It might be because of the slowed soil acidification, as  $\text{NH}_3$  was released when  $\text{CO}(\text{NH}_2)_2$  was hydrolyzed in soil (Guo *et al.* 2011), its less soil pH decrease compared with other N types supported our analysis (**Supplementary Online Material 8**).

Previous work has shown that when other nutrients were added in addition to N fertilizer, the effect sizes might change (Wang *et al.* 2018a). Among all N types, actually, total microbial biomass only increased after  $\text{Ca}(\text{NO}_3)_2$  addition (+3.66 %). This may be attributable to the alleviation of  $\text{Ca}^{2+}$  limitation (Treseder 2008), which microorganisms could more tolerant to greater N. Additionally, the base cations  $\text{Ca}^{2+}$  and  $\text{K}^+$  played critical roles in buffering against N-induced soil acidification, especially at the early stage (Tian & Niu 2015). Contrarily, total microbial biomass decreased the most after  $(\text{NH}_4)_3\text{PO}_4$  addition (-45.9 %,  $P < 0.001$ ). However, the data were extracted from only one observation, the result was less reliable. Although  $\text{KNO}_3$ ,  $\text{Ca}(\text{NO}_3)_2$ ,  $(\text{NH}_4)_3\text{PO}_4$  or  $(\text{NH}_4)_2\text{SO}_4$  were sometimes used as fertilizer in some field experiment, the added  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{PO}_4^{3-}$  or  $\text{SO}_4^{2-}$  with N fertilizers may mask, offset and even mislead the direct effects of N on soil microorganisms. Thus, they were not recommended as N fertilizers in field experiment. Contrarily,  $\text{NH}_4\text{Cl}$  and  $\text{NaNO}_3$  were suggested to be selected as  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$ , as  $\text{Cl}^-$  and  $\text{Na}^+$  was relative abundant in soil and microbial metabolism was less limited by them.

Fungi and bacteria are the main constituents of soil microbial community. The biomass of both fungi

and bacteria significantly decreased after N addition. Compared with fungal biomass, bacterial biomass less decreased, which indicated greater sensitivity of fungi to N addition (Zhang *et al.* 2017). In line with previous results,  $\text{NH}_4^+$ -N addition induced significant and dramatical decline in fungal and bacterial biomass compared with other N types, which also meant its serious negative impact on soil microorganisms. A higher fungi to bacteria (F/B) ratio indicates stronger soil ecosystem buffering capacity, better sustainability of soils and higher microbial metabolic efficiency (Chen *et al.* 2018). The decreased F/B ratio after N addition in this work indicated less sustainability of soil ecosystem. Furthermore, the ratio was sensitive to N type.  $\text{NH}_4^+$ -N addition led to significant and the largest F/B ratio decline than other N types, especially for  $(\text{NH}_4)_2\text{SO}_4$ , which also indicated its serious negative impact on the function and sustainability of soils. Compared with  $\text{NH}_4\text{NO}_3$ ,  $\text{CO}(\text{NH}_2)_2$  addition resulted in a more significant F/B ratio decline, which meant soil microorganisms may be more sensitive to  $\text{CO}(\text{NH}_2)_2$  (Guo *et al.* 2011; Du *et al.* 2014).

In soil microbial community, both actinomycete and saprophytic fungal biomass were less affected by N addition. Actinomycete is thought to be largely unaffected by soil pH (Rousk *et al.* 2010), the influence of acidification caused by N addition might be less obvious. The insignificant relationship between actinomycete biomass and soil  $\Delta\text{pH}$  supported our hypothesis ( $P = 0.439$ , **Supplementary Online Material 9**). Soil saprophytic fungi are primarily C limited (Bonner *et al.* 2019), the direct effects of different N types on them might be similar. Although no statistically significant differences were shown among different N types for them,  $\text{NH}_4^+$ -N addition resulted in the most serious biomass decline as well. The biomass of AM fungi declined the most in all types of microorganisms. When N was added, plant roots might assimilate these available N compounds directly and might not have to absorb them via mycorrhizal symbionts. It would finally lead to AM fungal biomass decline (Kong *et al.* 2018). Interestingly, the effect of  $\text{CO}(\text{NH}_2)_2$  addition on AM fungal biomass was similar to  $\text{NH}_4^+$ -N. When  $\text{CO}(\text{NH}_2)_2$  was fertilized, part of it will be directly absorbed by hyphae and then broken down to  $\text{NH}_4^+$ -N, while others will be hydrolyzed to  $\text{NH}_4^+$ -N in soil and then absorbed by hyphae (Govindarajulu *et al.* 2005). Thus,  $\text{CO}(\text{NH}_2)_2$  displayed a similar effect as  $\text{NH}_4^+$ -N. Although  $\text{NO}_3^-$ -N can also be taken up by AM fungal hyphae, it will be converted to  $\text{NH}_4^+$ -N (Hodge 2017). However, it is a high-energy demand process (Wang *et al.* 2015) and more AM fungi would be needed to translocate these massive  $\text{NO}_3^-$ -N.

$\text{G}^+$  and  $\text{G}^-$  bacterial biomass were less impacted by N addition. However, N addition induced an increased  $\text{G}^+/\text{G}^-$  ratio, especially after  $\text{CO}(\text{NH}_2)_2$  addition. A possible explanation is the reduced belowground C-allocation by the trees with increasing N loading (Wang *et al.* 2015). Additionally,  $\text{G}^+$  bacteria are more stress-tolerant than  $\text{G}^-$  bacteria (Wang *et al.* 2018b). In soils, higher  $\text{G}^+/\text{G}^-$  ratio indicates greater soil organic carbon accumulation (Zhang *et al.* 2015). The greatest  $\text{G}^+/\text{G}^-$  ratio increase after  $\text{CO}(\text{NH}_2)_2$  addition not only indicated a lower quality substrate but also an acclimation of microorganisms to changes in substrate and nutrient availability. Although  $\text{NH}_4^+$ -N addition induced significant  $\text{G}^+$  and  $\text{G}^-$  bacterial biomass and  $\text{G}^+/\text{G}^-$  ratio decline, they were derived from only one observation. We therefore do not have strong evidence to conclude that  $\text{NH}_4^+$ -N addition significantly affects  $\text{G}^+$  and  $\text{G}^-$  bacterial biomass.

The impacts of N addition on soil microbial biomass may also depend on the ecological factors, such as MAT, MAP, ecosystem type, N addition rate and application duration (Treseder 2008; Fu & Shen 2017; Wang *et al.* 2018a; You *et al.* 2018; Zhang *et al.* 2018; Jia *et al.* 2020). Because of low background levels of N deposition (Wardle *et al.* 2013), microorganisms of tundra ecosystem is the most sensitive to N and finally exhibit the most severe biomass decline when N was added. Compared with forest soils, microbial biomass of grassland soils decreased greater, which indicated its more sensitivity to N addition. It may be because of the higher C/N ratios and long lifetime of C-cycle of forest soils (Townsend *et al.* 1996). Similarly, microbial biomass of coniferous forest were more sensitive to N deposition than that of broadleaved forest for all N types. For all types of ecosystem,  $\text{NH}_4^+$ -N induced to the greatest biomass decline and  $\text{CO}(\text{NH}_2)_2$  induced to the smallest decline, which indicated the significant effects of different N on microbial biomass were consistent and commonly widespread across all ecosystem.

Although globally wide ranges of variations in MAT, the responses of microbial biomass to N addition did not change spatially. However, field experiment sites with higher precipitation may lead to less significant

soil microbial biomass decline, especially for  $\text{NO}_3^-$ -N (**Supplementary Online Material 10**). It may be due to its greater leaching than other N types. Some reports revealed that the responses of ecosystem to N deposition might be linear or nonlinear, such as linear (Gu *et al.* 2019) or exponentially increasing  $\text{N}_2\text{O}$  emissions (Shcherbak *et al.* 2014) and curve changing of soil respiration (Peng *et al.* 2017) to N. Our analysis showed that the general trend of soil microbial biomass to N addition is linear. In consistent to some reports (Humbert *et al.* 2016), soil microbial biomass decline was significantly correlated with the accumulated N amount (N addition rate  $\times$  application duration). It indicated that N addition with great rate and short duration will lead to similar microbial biomass changes as low amount for a long term, and the declining trends were consistent among all ecotypes. Among these N types,  $\text{NH}_4^+$ -N addition revealed the most serious soil microbial biomass decline, which indicated its more serious negative effect on soil microorganisms than other N types.

Actually, the deposited N from the atmosphere is mixed with different N types and the percentages of  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N and  $\text{CO}(\text{NH}_2)_2$  are significantly different in the world scale. In this work, soil microbial biomass significantly increased (+19.8 %) when mixed N ( $\text{NH}_4\text{NO}_3 + \text{CO}(\text{NH}_2)_2$ ) was fertilized. However, the data were extracted from only two articles. Less data were not enough to support the conclusion that mixed N addition significantly accelerated soil microbial biomass. Whether mixed N fertilization will display significantly different effects on the soil microorganisms from single N type should be further investigated. All the results suggested that when we estimate the effects of N deposition on ecosystem, the type of N fertilizer should be concerned, rather than only focus on the total N deposition amount. In addition, the natural deposited N components of sample site should also be concerned and investigated before experiment.

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**Table 1** The influences of addition with different N types on the changes (%) of soil microbial characteristics under different ecosystems.

Ecotype	Total N	NH <sub>4</sub> <sup>+</sup> -N	NO <sub>3</sub> <sup>-</sup> -N	NH <sub>4</sub> NO <sub>3</sub>	CO(NH <sub>2</sub> ) <sub>2</sub>
Forest	-6.90*** A	-19.1*** a A	-9.81*** ab A	-4.94** b A	-7.37* b A
Broadleaved forest	-2.70* A	-10.7* a A	-8.91 ab* AB	-2.70 ab B	8.46 b A
Coniferous forest	-3.51* A	-22.3*** a A	-12.1*** a A	-7.44** b C	-10.1** a A
Grassland	-10.9*** B	-35.8*** a B	-3.61* b B	-10.1*** b B	-9.07*** b A
Tundra	-11.5** AB	-31.1*** a AB	-4.29 b AB	-8.92* b A	N/A
Shrub	-4.77* ABD	N/A	-2.36 a AB	-5.38* a AB	N/A
Desert	3.15D	N/A	N/A	3.15 A	N/A
Wetland	12.9* CD	N/A	N/A	12.9* C	N/A

\*, \*\*, and \*\*\* indicate significant differences at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$  respectively.

ANOVA was used to compare the effects of different N application on soil microbial biomass under different ecosystems and multiple comparisons were performed using the LSD method. The small letters in rows are the comparison of the effects of different N types on soil microbial biomass for each ecotype; the capital letters in columns are the comparison of soil microbial biomass in different ecotypes for each N type.

The same letter means no statistically significant difference among the treatments under the level of  $P < 0.05$ .

The details were shown in **Supplemental Information 7**.

## Figure Legends

**Fig. 1** Influence of addition with different types of N fertilizers on soil total microbial biomass.

Effect sizes are natural log response ratios ( $\ln RR$ ), with positive and negative values indicating a positive and negative effect of N addition respectively. Points represent means ( $\ln RR$ ) and error bars present 95 % confidence intervals (CI). The number in parentheses next to the bar represents the number of observations. The same below.

**Fig. 2** Influence of addition with different types of N fertilizers on soil microbial characteristics.

**A**, Fungal biomass; **B**, Bacterial biomass; **C**, Fungi to bacteria (F/B) ratio; **D**, Actinomycete biomass; **E**, Saprophytic fungal biomass; **F**, Arbuscular mycorrhizal (AM) fungal biomass; **G**, Gram-positive ( $G^+$ ) bacterial biomass; **H**, Gram-negative ( $G^-$ ) bacterial biomass; **I**,  $G^+/G^-$  ratio.

The details of the analysis were shown in **Supplementary Online Material 6**.

**Fig. 3** Relationship between the changes of microbial characteristics ( $\ln RR$ ) and accumulated N amount (N addition rate  $\times$  application duration).

**A**,  $NH_4^+-N$ ; **B**,  $NO_3^--N$ ; **C**,  $NH_4NO_3$ ; **D**,  $CO(NH_2)_2$

### Supplementary Online Material Legends

**Supplementary Online Material 1** Data extracted from 178 papers.

**Supplementary Online Material 2** List of 178 papers used in the meta-analysis.

**Supplementary Online Material 3** Global distribution of the 178 field experiments sites around the world.

**Supplementary Online Material 4** Relationship between soil microbial characteristics ( $\ln RR$ ) and other factors.

**Supplementary Online Material 5** Meta-Analysis Code.

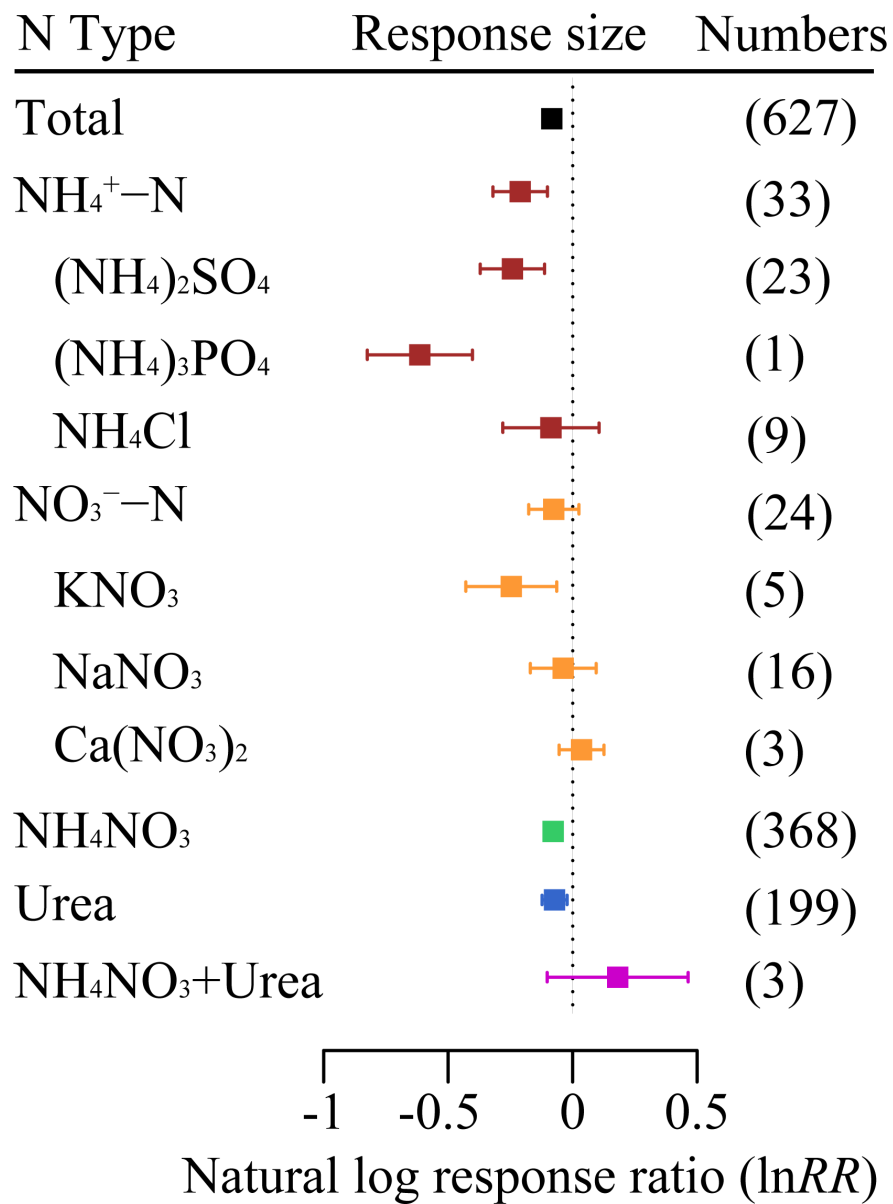
**Supplementary Online Material 6** Influence of addition with different types of N fertilizers on soil microbial characteristics.

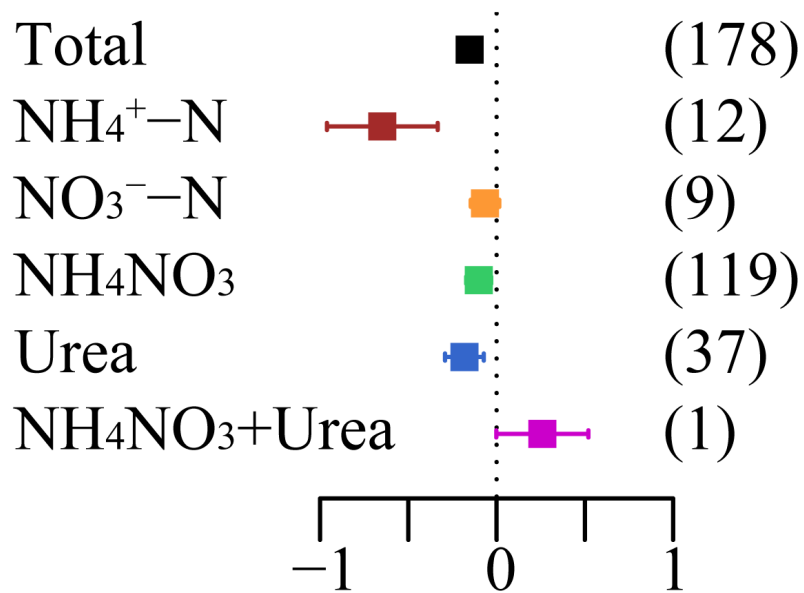
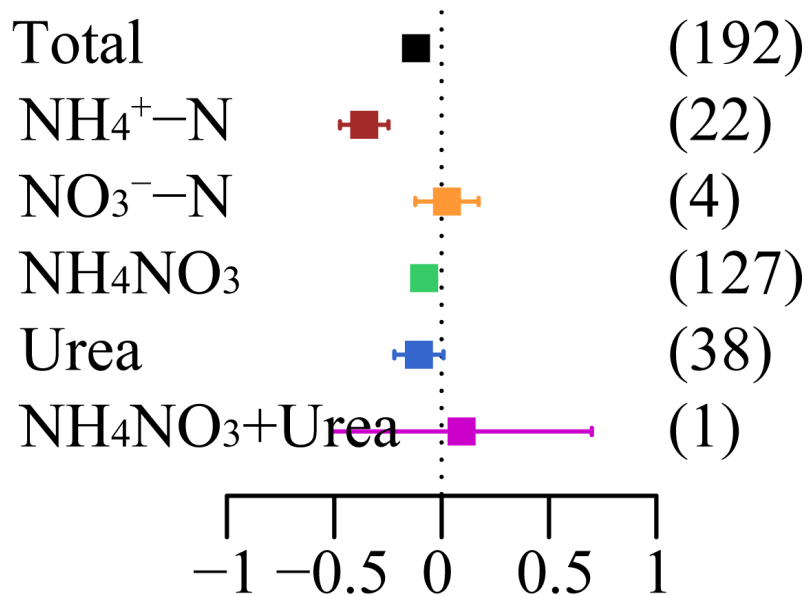
**Supplementary Online Material 7** Detailed information of the influence of addition with different N types on soil microbial characteristics under different ecosystems.

**Supplementary Online Material 8**. Relationship between N fertilized rate and  $\Delta pH$ .

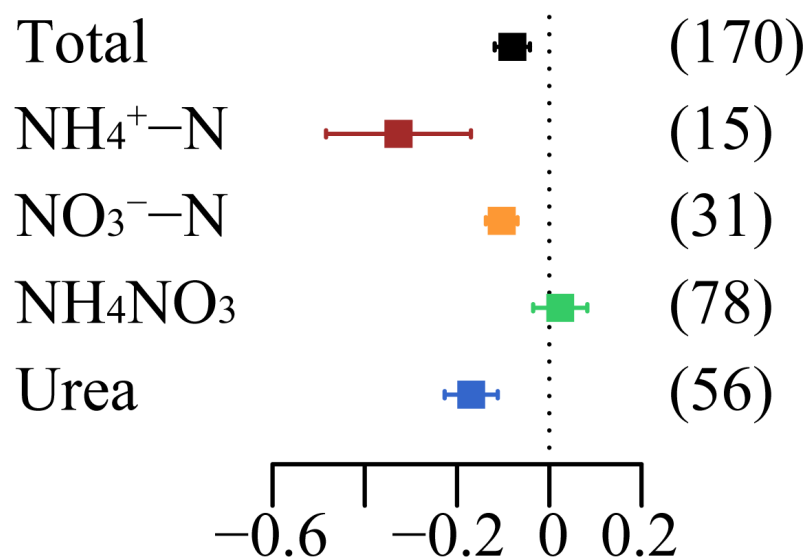
**Supplementary Online Material 9** Relationship between actinomycete biomass and  $\Delta pH$ .

**Supplementary Online Material 10** Effects of MAP on the total microbial biomass after addition of different N types.

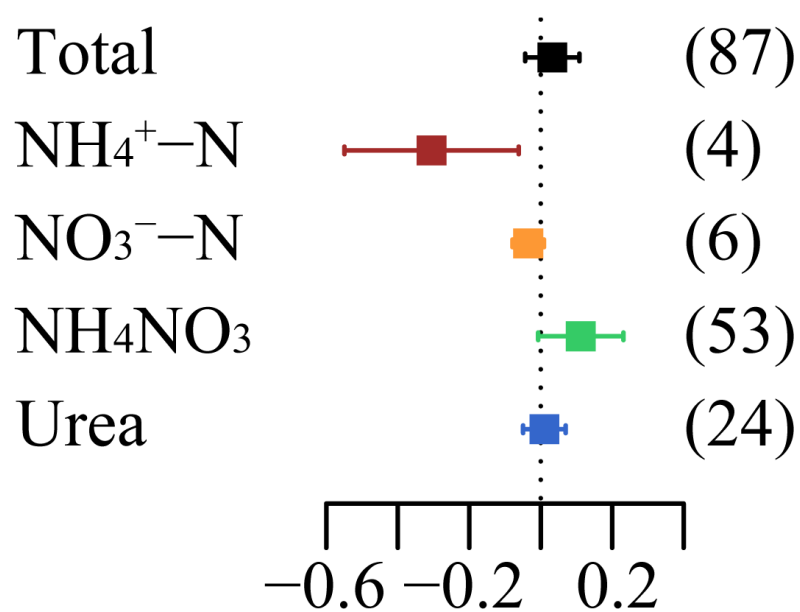


**A****B**

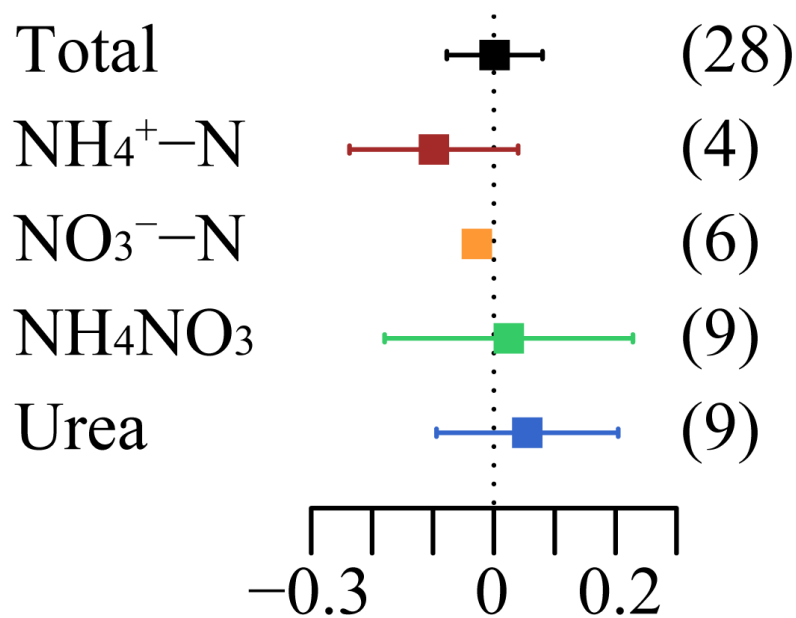
**C**



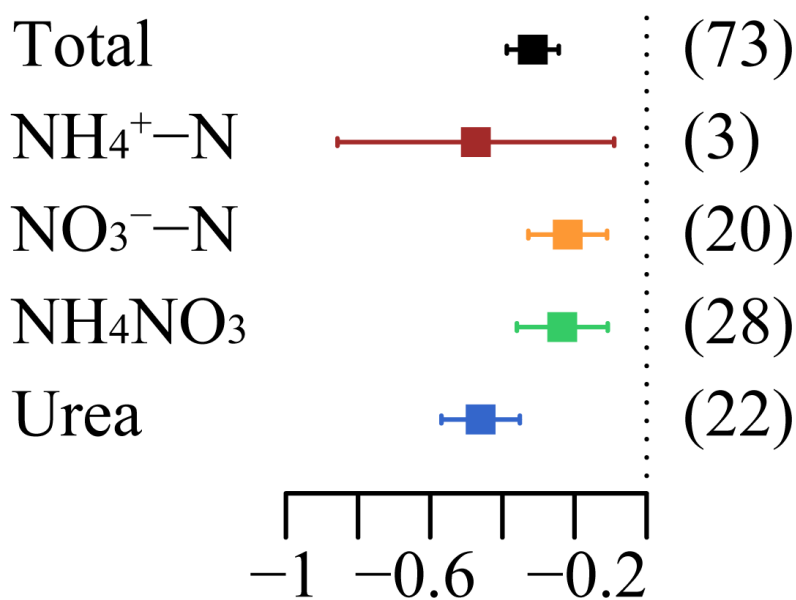
**D**



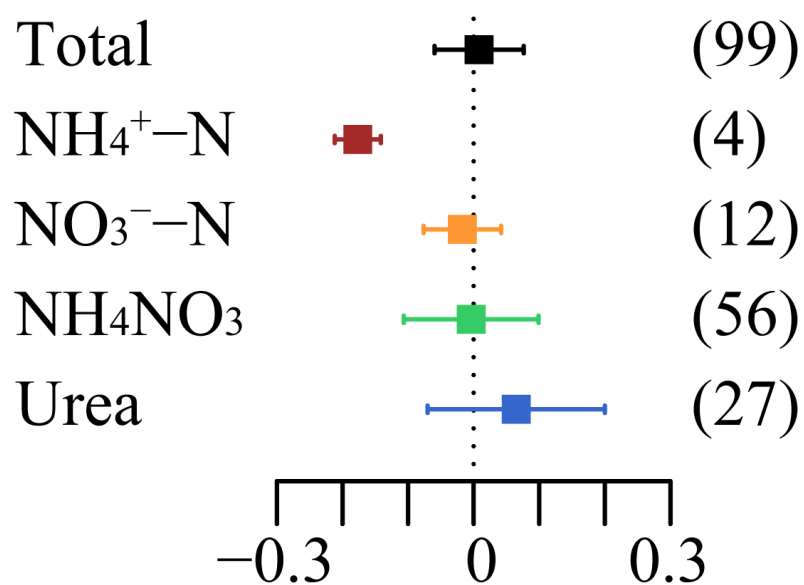
# E



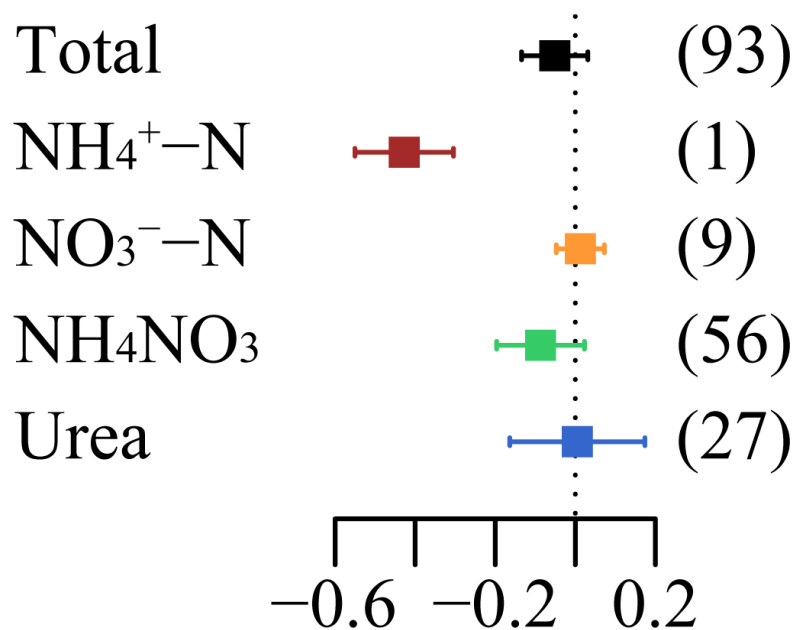
# F



# G



# H





# I

