

Temporal variability of ammonia emission potentials for six plant species in an evergreen subtropical forest in southwest China

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Abstract The temporal variability of leaf ammonia (NH₃) emission potentials (the ratio of leaf tissue ammonium to proton concentration) and nitrogen (N) pools of six dominant plant species were investigated at the Tieshanping (TSP) forested catchment, southwest China. The results showed that the NH₃ emission potentials and N pools presented small variations among seasons, which were mainly controlled by plant species and the leaf age. Also, high emission potential in one species did not correspond to high tissue N content. Specifically, the Chinese fir (*Cunninghamia lanceolata*) had higher NH₃ emission potential (mean: 46.2) but lower N content (mean: 1.6% of Dw). The leaf privet (*Ligustrum quihoui* Carr.) was with the moderate emission potential (15) and the highest N content (2.7% of Dw) on average, which for the Masson pine (*Pinus massoniana*) were both low. Overall, the emission potentials of the six species were too low (<200) to build up a sufficiently high NH₃ partial pressure in the leaves. Therefore, the Masson pine dominant subtropical

forest at TSP acts as a sink for the atmospheric NH₃, indicating that using the N flux in throughfall only may significantly underestimate the N income of the ecosystem. The results are informative for future modeling of plant-atmosphere NH₃ exchange and estimating N budget in local or regional scales.

Keywords Ammonia · Emission potential · Temporal variability · Nitrogen pool · Subtropical forests

Introduction

Forest canopies and forest ecosystems are considered to be nitrogen (N) limitation and not N excess in pre-industrial times (Rennenberg et al. 1998). However, due to the increase use of fossil fuels and expansion in agriculture, many forests in China are exposed to excessive amounts of N (Chen and Mulder 2007). Ammonia (NH₃) accounts for almost half of the reactive N pollutants in the atmosphere with wide ecological and environmental impacts, including eutrophication, acidification, alteration of biodiversity, and global warming (Allen et al. 2011; Beusen et al. 2008; Krupa 2003; Stamenkovic et al. 2015; Sutton et al. 2008; Wang and Schjoerring 2012). In China, the total NH₃ emission was estimated to be 13.6 Tg in 2000, 50% of which came from fertilizer applications and 38% from other agricultural sources. In recent years, different values of NH₃ emission estimation have been reported with some uncertainties, e.g., 16.1 Tg by Dong et al. (2010) and 9.8 Tg by Huang et al. (2012) in 2006.

Forest vegetation often acts as a sink for the atmospheric NH₃ (Hansen et al. 2013; Neiryneck et al. 2005). However, owing to the existence of stomatal NH₃ compensation point (χ_s) in plant leaves, the exchange between the atmosphere and the canopies is in fact bi-directional (Flechard et al. 2013). Episodes of NH₃ emission may occur when the air concentration of NH₃ goes

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below the χ_s (Geßler et al. 2000; Neiryck and Ceulemans 2008; Zhang et al. 2010). Canopies exposed to high atmospheric N deposition are more likely to emit NH_3 (Wang et al. 2013).

In the absence of χ_s , the ratio between ammonium (NH_4^+) and proton (H^+) concentration of leaf extracts is calculated to represent the potential NH_3 emission designated Γ , although the parameter is not a direct measure of the NH_3 exchange between plants and the atmosphere (David et al. 2009; Mattsson and Schjoerring 2002; Mattsson et al. 2009). Γ is temperature independent and dimensionless, and therefore often more convenient to use than χ_s (Nemitz et al. 2000a, b). Clear seasonal variations of Γ for a beech forest, with peak values at autumn, have been found by Wang et al. (2011) in Denmark. The Γ value alters across plant species such as in managed grasslands (Mattsson et al. 2009) and in forests (Wang et al. 2013). Within the canopy, different tissues (related to different growing stages) of the plant contribute differently to NH_3 flux potentials (Herrmann et al. 2009). The senescent and fallen leaves are recognized as the strongest source, whereas green leaves may recapture NH_3 emitted in deeper layers of the canopy (David et al. 2009; Wang and Schjoerring 2012).

For the high applications of N fertilizer, the Γ values of grass or crops have drawn much attention (David et al. 2009; Mattsson and Schjoerring 1996; Mattsson et al. 2009; Nemitz et al. 2000a; van Hove et al. 2002). In contrast, only sporadic works were conducted in temperate forest areas with a narrow range of plant species (Neiryck and Ceulemans 2008; Wang et al. 2011; 2013). Limited information is available on Γ values and N pools of plants in subtropical forests. To the best of our knowledge, parameterization of bidirectional NH_3 exchange between forests and the atmosphere is mainly based on datasets from temperate locations in northern Europe, and there exists large uncertainties when applied to tropical and subtropical areas (Massad et al. 2010).

Therefore, the primary objectives of this work are (1) to investigate the seasonal variations of NH_3 emission potentials and N pools for six dominant plant species at the Tieshanping forested catchment, southwest China; (2) to assess the effect of different leaf ages on the NH_3 emission potentials and N pools within the canopies; and (3) to identify the subtropical forest in this catchment acting as a sink or source for the atmospheric NH_3 preliminarily. These will be quite valuable for modeling the plant-atmosphere NH_3 exchange and estimating N budget in local and regional scales, as well as assessing the ecological and environmental functions of subtropical forests on the reactive N pollutants.

Material and method

Site description

Tieshanping (TSP) (29°38'N, 106°41'E) is a 16.3-ha forested catchment, located about 25 km northeast of Chongqing,

southwest China. It is typically subtropical monsoonal climate with a mean annual temperature of 18.2 °C, and the total annual rainfall is 1028 mm (3-year averages, 2001–2003). The inorganic N deposition was 41 kg N ha⁻¹ year⁻¹, 61% of which occurred in the form of ammonium (NH_4^+ -N) (Chen and Mulder 2007). The soil type is a clay-rich loamy yellow mountain soil classified as Haplic Acrisol (WRB 2006) developed from sandstone. The catchment is an evergreen coniferous-broad-leaved mixed forest planted since the 1960s, dominated by Masson Pine (*Pinus massoniana*) and some associated species such as Chinese fir (*Cunninghamia lanceolata*), Camphor (*Cinnamomum camphora*), and Schima (*Schima superba* Gardn. et Champ). Below the tall arbor trees, well-developed understory of evergreen shrubs exist (Chen and Mulder 2007; Zhu et al. 2013). The average forest stand stocking in 2002 was about 1095 trees ha⁻¹, and the average height and mean diameter at breast height of Masson pine (Kraft class 1–3) were 15.5 m and 193 mm, respectively (Jiang and Zhang 2008; Wang et al. 2007).

The atmospheric concentration of NH_3 was measured with denuders in the forest according to Lun et al. (2003) and Nie et al. (2010). The seasonal mean air concentrations of NH_3 were 2.56, 2.89, 1.45, and 1.91 nmol mol⁻¹ in spring, summer, autumn, and winter, respectively.

Sample collection

In this study, six dominant plant species from the arbor layers to the understory were investigated, i.e., the Masson pine (Pine), Camphor, Chinese fir (Fir), Schima, Leaf privet (*Ligustrum quihoui* Carr.), and Woodwardia (*Woodwardia japonica* (L. f.) Sm.). More information about the six species is shown in Table 1. The leaf privet and Woodwardia were abbreviated as Privet and Fern hereafter, respectively.

For each species, two plants at the similar growing status were chosen and labeled. Three small twigs (30–40 cm long, base canopies) were cut off from each of the pre-labeled plant, and then collected the leaves or needles excluding the petioles along the leaf edge. The air temperature and the relative humidity were recorded for two times on the sampling day, i.e., at the beginning and at the end of the sample process (lasting for about 2 h). The average of air temperatures and the mean relative humidity within each of the sampling process are shown in Fig. 1, as well as the monthly mean temperature and the total precipitation during the experiment period (from June 2014 to May 2015).

All the leaves or needles were gently washed with distilled water and divided into two sub-samples. One sub-sample was frozen in liquid nitrogen immediately and thereafter stored in a refrigerator (−80 °C) before determination of the leaf tissue chlorophyll concentration, NH_4^+ concentration, and pH. The second sub-sample was dried for analyzing the relative water content, total nitrogen content (TN), and total carbon content (TC) of leaves or needles.

Table 1 Basic information about the six experimental plant species

Plant species	Latin name	Leaf habits	Stand structure
Masson pine	<i>Pinus massoniana</i>	Evergreen conifer	Macrophanerophytes
Camphor	<i>Cinnamomum camphora</i>	Evergreen broad-leaved	Macrophanerophytes
Chinese fir	<i>Cunninghamia lanceolata</i>	Evergreen conifer	Mesophanerophytes
Schima	<i>Schima superba</i> Gardn. et Champ.	Evergreen broad-leaved	Mesophanerophytes
Leaf privet	<i>Ligustrum quihoui</i> Carr.	Semi-evergreen broad-leaved	Shrub
Woodwardia	<i>Woodwardia japonica</i> (L. f.) Sm.	Evergreen	Pteridophyte

Notes: The Leaf privet is a deciduous or semi-evergreen plant, and at the Tieshanping catchment, it shows the characteristics of semi-evergreen plant

Experimental design

Two trials were designed to investigate the seasonal variations of NH_3 emission potentials and N pools for the six dominant plant species (trial 1) and to understand the effect of leaf age on the NH_3 emission potentials and N pools (trial 2).

Trial 1: variations among seasons and species

Trial 1 and trial 2 were conducted from June 2014 to April 2015 (between 9:00 am and 15:00 pm). In each season, at least three times of sampling were set up, and the sample intervals were half a month. Mature leaves or needles (fully developed green leaves or needles) of the six species were collected from the pre-labeled plants. Leaves or needles from the same plant were pooled into one sample.

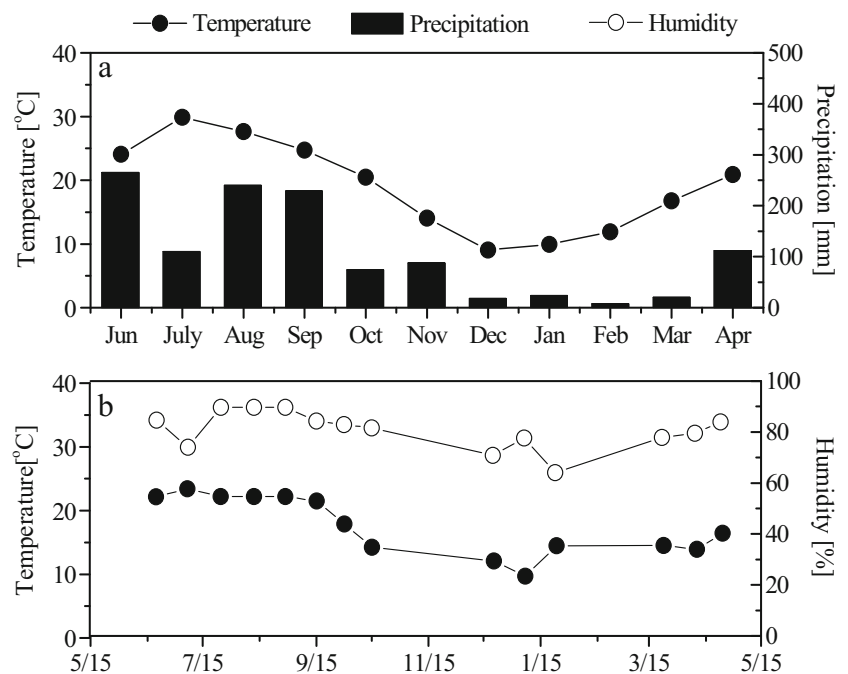
Trial 2: variations among different leaf ages

Leaves or needles with different ages of the Schima, Camphor, Fir, and Pine were chosen as the experimental materials in this trial. Specifically, the leaves or needles were divided into four ages, including new green leaves (just budding or developing light green leaves, if present), mature leaves, senescent leaves (attached leaves with visual symptoms of senescence, if present), and fallen leaves (senescent or dead detached leaves). The fallen leaves were collected by nylon nets fixed under the labeled trees. For each leaf age, leaves or needles from the same labeled tree were mixed into one sample.

Sample analysis

Frozen leaf or needle materials were extracted and thoroughly grounded in a cooled mortar by 10 mM formic acid for bulk

Fig. 1 The monthly mean temperature and the monthly total precipitation during the experiment period (from June 2014 to May 2015) (a), as well as the averages of air temperature and the mean relative humidity during each of the sampling process (lasting for about 2 h) (b)



tissue NH_4^+ concentration and by distilled water for bulk tissue proton activity ($\text{H}^+ = 10^{-\text{pH}}$) (leaf to water ratio, 1:10). Thereafter, the suspensions were centrifuged for 10 min (10,000g, 4 °C), and the supernatants were collected and filtered (using 0.45 μm syringe filter) for analysis. Details of the analytical procedures can be obtained from Mattsson et al. (2009) and Wang et al. (2011). The NH_4^+ concentration was analyzed by a continuous flow-injection analyzer (San⁺⁺, Skalar, The Netherlands). The tissue pH was determined by a pH meter (PHB-4).

Frozen materials were extracted by 96% (v/v) alcohol for analysis of tissue chlorophyll concentration (both chlorophylls a and b) by an ultraviolet-visible spectrophotometer (UV-5100) (Zou 2000). The relative water content (% of Fw) was obtained through drying fresh leaves or needles for 48 h at 70 °C. After that, the dried leaves or needles were grounded and analyzed for leaf TN and TC contents (% of Dw) using an element analyzer (Vario EL III, Elementar Analysen systeme GmbH, Germany).

The calculation of NH_3 emission potential

NH_3 emission potential (Γ) was determined by using the leaf tissue NH_4^+ and H^+ concentration (Mattsson and Schjoerring 2003; Wang et al. 2011) according to the equation:

$$\Gamma = \frac{[\text{NH}_4^+]}{[\text{H}^+]}$$

where $[\text{NH}_4^+]$ and $[\text{H}^+]$ are the NH_4^+ concentration and the proton activity ($[\text{H}^+] = 10^{-\text{pH}}$) in the leaf extracts, respectively.

Data analysis

Data were computed and analyzed by Microsoft Excel 2013 and SPSS 17.0 with significant differences at $p < 0.05$ (one-way ANOVA, LSD) unless otherwise stated. Pearson’s correlation analysis was used to gain the correlations between leaf NH_3 emission potentials, N pools, and the effecting factors. Figures in the paper were drawn by Sigma plot 10.0 and Origin 8.0.

Results

Eight parameters were referred to, i.e., the leaf tissue NH_4^+ concentration, pH, Γ , N content, the ratio of TC to TN (C/N ratio), the concentration of chlorophyll (Chl (a + b)), the ratio of chlorophyll a to chlorophyll b (Chl a/b), and the relative water content. The leaf tissue NH_4^+ concentration and N content represent the N status of plants, and Γ value is a simple indicator to assess plants’ ability or potential to emit NH_3 to the atmosphere. The bigger the Γ is, the more possibility of

gaseous NH_3 release from the plants to the air. The Chl (a + b) and Chl a/b ratio are useful indicators of the physiological activity and the degree of senescence. When the leaves senesce, the values of Chl (a + b) and Chl a/b ratio may sharply decline.

Variations among seasons and plant species

Leaf tissue NH_4^+ concentration, pH, and NH_3 emission potential

The average leaf tissue NH_4^+ concentrations of the six species presented small seasonal variations, which were 89.2, 101.6, 85.5, and 103.7 μM in spring (March to April), summer (June to August), autumn (September to October), and winter (December to January), respectively (Fig. 2a). For the plants Schima, Camphor, Fern, and Fir, the tissue pH (Fig. 2b) in spring and winter (average: 5.1) was generally higher than that in summer and autumn (average: 4.9). Consequently, the mean Γ value of these species showed a slight increase in spring (20.1) and winter (32.0), corresponding to 10.1 and 15.3 in summer and autumn, respectively (Fig. 2c).

The variations of tissue NH_4^+ concentration and pH among species were both significant. The Fir needles had the largest NH_4^+ concentration (mostly $>200 \mu\text{M}$), and nearly one order of magnitude higher than that in Schima and Camphor leaves. The average tissue NH_4^+ concentrations of Schima, Camphor, Fern, Privet, and Pine ranged from 17.7 to 49.0 μM , differences among which were insignificant (Fig. 2a). The tissue pH of Schima and Pine was normally less than 4.6 (Fig. 2b) and clearly lower than that in the leaves or needles of the other four species (mostly >5). During spring, the average tissue pH in the Fir needles occupied 92.4 and 87.9% of that in the Fern and Privet leaves, during summer which accounted for 97.1 and 84.0% of that in the Fern and Privet leaves, respectively.

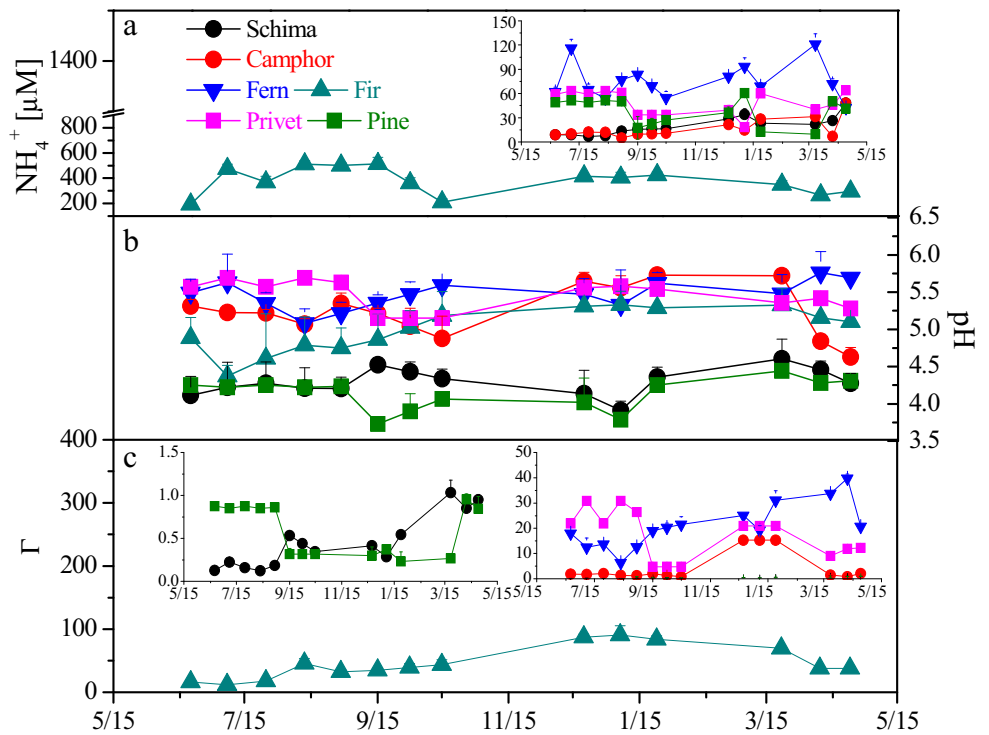
Clear variability of Γ was observed among species (Fig. 2c). Generally, the Fir needles had the largest Γ values (mostly >30) and significantly larger than those for the other five species. The Fern and the Privet were often the second and the third in Γ values (about 20 and 15, respectively), the differences between which were normally significant. The Schima and the Pine leaves or needles mostly had small and comparable Γ values (<2).

Chlorophyll

The mean Chl (a + b) concentrations were 1.2, 1.4, 1.3, and 0.93 mg g^{-1} Fw in spring, summer, autumn, and winter, respectively (Fig. 3a). The Chl a/b ratios of the six species during the four seasons were in the ranges of 1.4–2.2, 1.3–2.1, 1.3–2.4, and 1.3–2.3, respectively (Fig. 3b).

Both the variations of Chl (a + b) concentration and Chl a/b ratio among plant species were obvious. The mean Chl (a + b)

Fig. 2 Seasonal variations of leaf tissue NH_4^+ concentrations (a), pH (b), and NH_3 emission potentials (c) of the six plant species

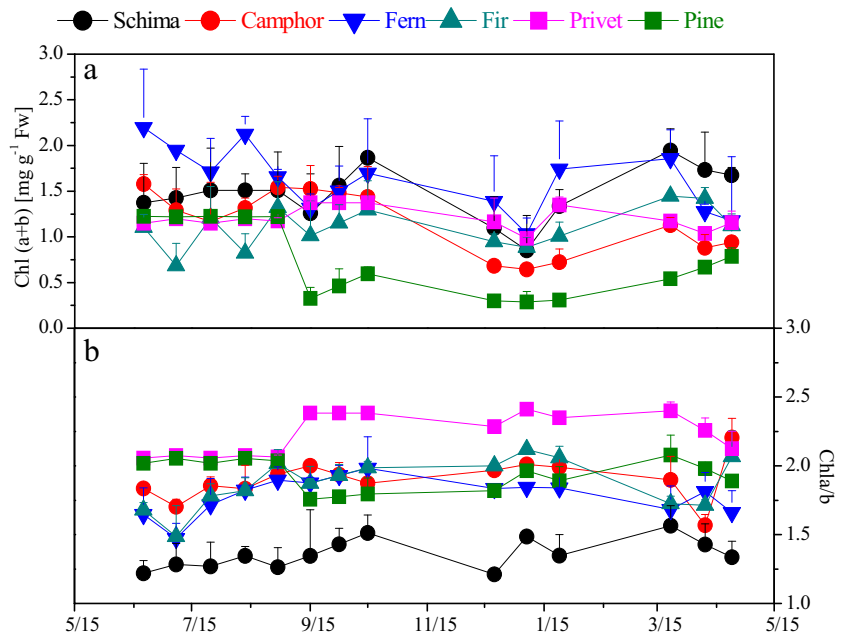


Notes: The values are means ($n = 2$), and the error bars represent standard errors.

concentrations of the six species could be divided into four classes, and the Fern leaves (the first class) had the largest Chl (a + b), i.e., $1.6 \text{ mg g}^{-1} \text{ Fw}$. The second class contained the Schima and Privet, both having the concentrations of

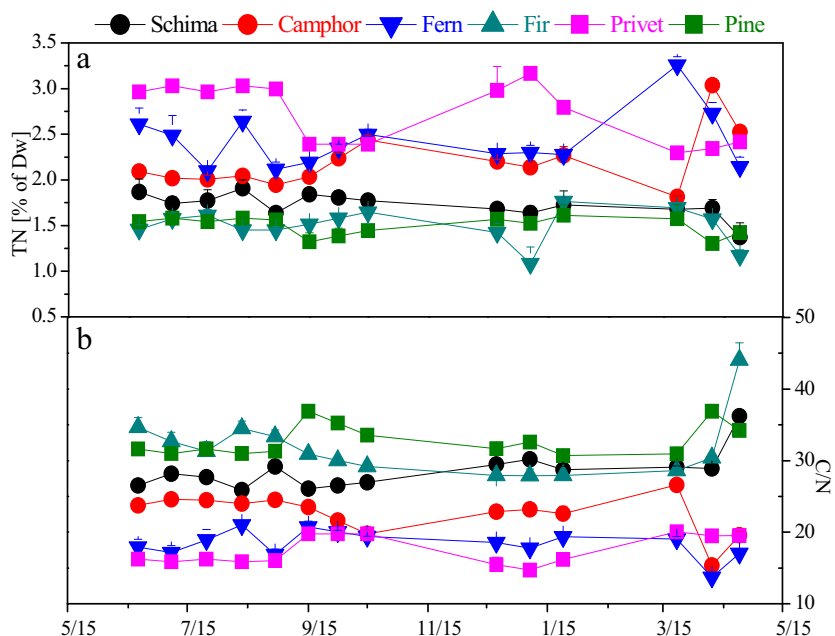
$1.3 \text{ mg g}^{-1} \text{ Fw}$. The third class included Camphor and Fir ($1.1 \text{ mg g}^{-1} \text{ Fw}$ on average). Pine was the last class with the concentration of $0.67 \text{ mg g}^{-1} \text{ Fw}$. Differences between the last class and the other three ones and between the first and the

Fig. 3 Seasonal variations of leaf tissue chlorophyll (a + b) concentrations (a) and chlorophyll a/b ratios (b) of the six plant species



Notes: The values are means ($n = 2$), and the error bars represent standard errors.

Fig. 4 Seasonal variations of leaf tissue N contents (a) and C/N ratios (b) of the six plant species



Notes: The values are means (n = 2), and the error bars represent standard errors.

third class were both significant. The averages Chl a/b of the six species were in the range of 1.4–2.3, and the Privet leaves had the highest ratio, which was significantly higher than that in the Schima. The Chl a/b ratios in the other four species were in moderate and comparable.

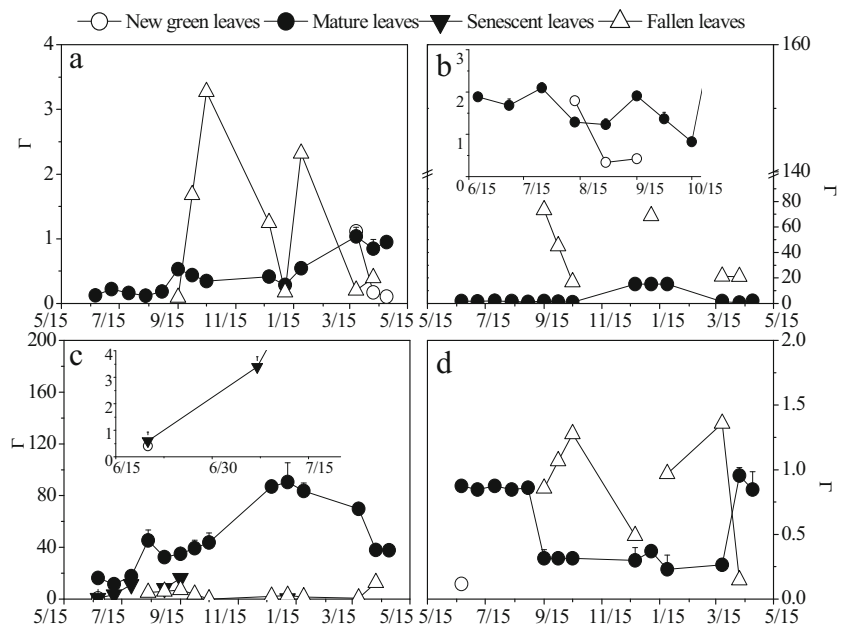
N content and C/N ratio

The average of tissue N contents for the six plant species were 2.1, 2.0, 1.9, and 2.1% of Dw in spring, summer, autumn, and winter,

respectively (Fig. 4a). The average C/N ratios of the six plants in autumn and winter were 8.2 and 7.4% lower than those in spring and 5.7 and 4.9% lower than summer (Fig. 4b).

Variations of tissue N content and C/N ratio were mostly significant among species, and the Fern and the Privet leaves had higher N content on average (2.4 and 2.7% of Dw, respectively) (Fig. 4a). Contrarily, the Fir and the Pine needles had lower mean N contents, i.e., 1.6 and 1.5% of Dw, respectively. The Schima and the Camphor leaves had the moderate mean N contents, i.e., 1.7 and 2.3%, respectively. Correspondingly, the

Fig. 5 The effect of leaf age on NH₃ emission potentials of Schima (a), Camphor (b), Fir (c), and Pine (d)



C/N ratios of the leaf tissue were higher in the Fir and Pine (mostly >30), lower in the Fern and Privet (mostly <20) (Fig. 4b), yet moderate in the Schima and Camphor.

Effects of leaf age on NH_3 emission potential, N content, and C/N ratio

NH_3 emission potential

Γ values of the Schima, Camphor, Fir, and Pine trees with different leaf ages are shown in Fig. 5. The average Γ values for fallen leaves or needles of the Schima, Camphor, and Pine were 149, 807, and 86.1% higher than the corresponding mature parts (Fig. 5a, b, d). The new green leaves or needles sometimes had lower Γ than those at the mature stage, but this pattern was not consistent. The mean Γ values (46.2) in the mature Fir needles were significantly higher than those in the new green, the senescent, and the fallen parts (Fig. 5c), which in the later three were comparable and without significant differences, i.e., 0.41, 6.7, and 4.1, respectively.

N content and C/N ratio

The average N contents of the fall leaves or needles of the Schima, Camphor, and Pine were 1.1, 1.8, and 1.1% of Dw, respectively, and clearly lower than those in the mature parts (1.7, 2.3, and 1.5%, respectively) (Fig. 6a, b, d). The new green Camphor leaves had the highest N content among the three ages, i.e., 2.6% on average (Fig. 6b).

Contrary to the N distribution during different leaf age, the fallen leaves or needles of the Schima, Camphor, and Pine

(Fig. 7a, b, d) had the largest mean C/N ratios (39.9), which were 47.0 and 53.7% larger than those in the mature and the new green parts, respectively. The difference between the new green and the mature leaves or needles of these species was negligible, and the lowest C/N ratio (15.5) was observed in the new green leaves of Camphor during spring (Fig. 7b). The variations of N content and C/N ratio among different Fir needle ages were small (Figs. 6c and 7c).

Discussion

In this study, temporal variations of Γ values and N pools were assessed for the six dominant plant species grown in a subtropical forested catchment, southwest China. Meanwhile, the effects of leaf age on them were also investigated. Consequently, we could identify the source or sink functions of plant canopies on the atmospheric NH_3 at the catchment preliminarily.

The Γ values for the mature leaves or needles of the six plant species ranged from 0.12 to 90.5 in this study (Fig. 2c), which were far smaller than those observed in grass or crop species (Mattsson and Schjoerring 2003; Sutton et al. 2001), and yet comparable to those recorded in temperate forests, i.e., 20–120 for Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) and 10–40 for Scots pine (*Pinus sylvestris* L.) needles (Wang et al. 2013). In the present study, the tissue NH_4^+ concentration and N content of the six species showed small seasonal variations (Figs. 2a and 4a). The Γ value of the Schima, Camphor, Fern, and Fir during spring and summer presented a little increase with a slight rise of tissue pH in these two

Fig. 6 The effect of leaf age on tissue N contents of Schima (a), Camphor (b), Fir (c), and Pine (d)

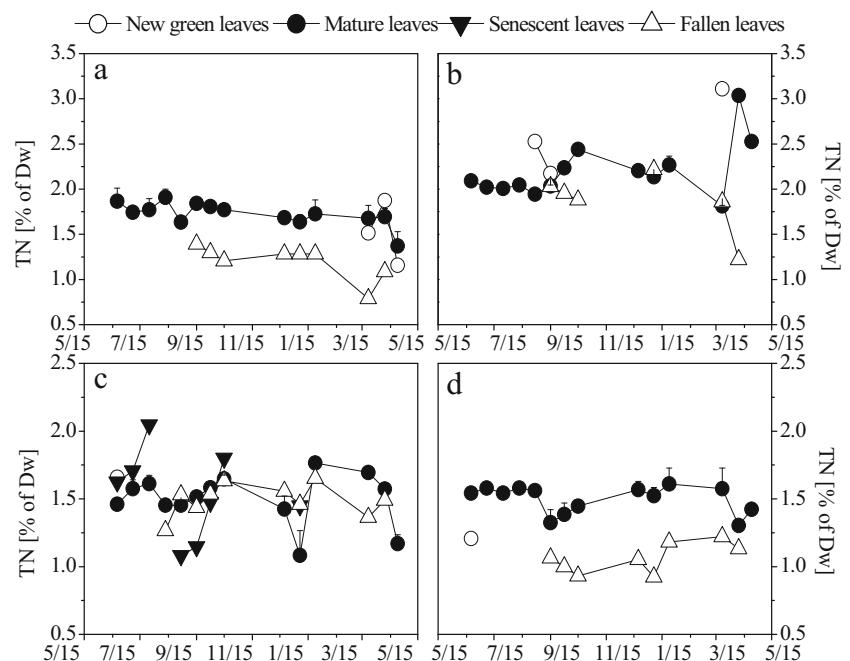
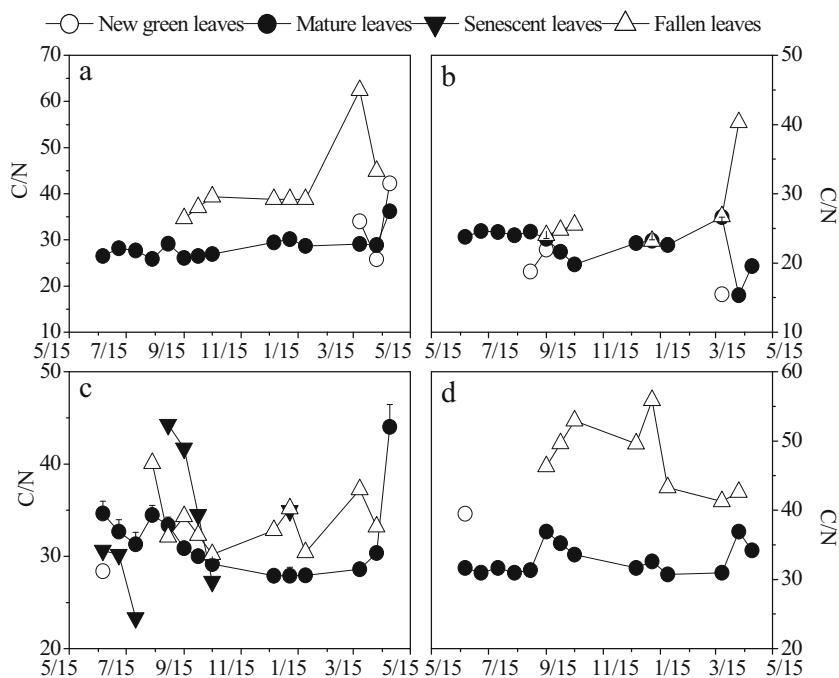


Fig. 7 The effect of leaf age on C/N ratios of Schima (a), Camphor (b), Fir (c), and Pine (d)



seasons (Fig. 2b, c). However, compared to the clear variability of Γ value among different leaf ages and plant species, this limited increase seemed negligible. In comparison with the deciduous, the evergreen species tended to retain much more foliage N within plants and therefore presented smaller seasonal fluctuation in N pools (Wang et al. 2013). These could be used to explain the unclear variations of foliage Γ values and N pools among seasons in this study, because the six species were all evergreen (Table 1). In addition, the fluctuations of air temperature in temperate forests throughout the whole year reported by Wang et al. (2013) were more than 25 °C with minus temperature in winter. Contrarily, in a subtropical forest in this study, the difference in air temperature between the hottest sampling day and the coldest was 13.7 °C with a minimum temperature of about 10 °C (Fig. 1b). We supposed that this range of temperatures was not low enough to lead to leaf senescence since small fluctuations of chlorophyll concentration and ratio in time scales were observed in the present study (Fig. 3 and Fig. S4c, d), which may probably result in the small variability of Γ values and N pools among seasons. Other possible explanation for the small temporal variability of foliage Γ values and N pools might be the low forest health status at the TSP catchment, especially the high defoliation percentage and mortality of Masson pine, which may in turn lead to the low transformation rate of N nutrients within the plants (Li et al. 2014; Wang et al. 2007).

As mentioned above, the variability of Γ values of mature leaves or needles between different plant species in this study was clear in all seasons (Fig. 2c), whereas this differences were not linked to the air concentrations of NH_3 which

fluctuated in a small range during the experiment (1.91–2.89 nmol mol^{-1}) at the TSP catchment. The significant variability in Γ values may affect by a range of species-dependent physiological processes involved in N uptake and N turnover in the leaves, which also reflect the foliage N status and the distribution of these two parameters across the plant species. In the present study, higher Γ value in one species did not correspond to higher N content, and Pearson’s correlation analysis showed that leaf tissue NH_4^+ concentration and N content were negatively correlated ($r = -0.16$, $p = 0.005$). This suggested that the N content in leaf could not be used to assess the plants’ potential to emit NH_3 at TSP, and a similar conclusion was obtained in an intensively managed grassland by Van Hove et al. (2002).

Leaf senescence represents the final stage of leaf development and is characterized by the transition from nutrient assimilation to nutrient remobilization (Masclaux et al. 2000). NH_4^+ is re-assimilated into amino acids when exported from the senescing leaves and may also be lost through leaching or volatilization of gaseous NH_3 (Schjoerring and Mattsson 2001). During senescent, the massive release of NH_3 was synchronized with protein and chlorophyll degradation (Mattsson and Schjoerring 1997; Schjoerring et al. 1998). In this study, both the Γ value and N content varied among different leaf ages (Figs. 5 and 6), and the new green leaves or needles of Schima, Camphor, and Pine normally had lower Γ . Furthermore, the Γ values in the fallen leaves or needles of these species were generally higher than those at the mature period, which were in line with the findings in senescent ryegrass leaves reported by Mattsson and Schjoerring (2003)

in a laboratory experiment and by Wang and Schjoerring (2012) in a field study. Meanwhile, the tissue Chl (a + b) concentration and the Ch a/b ratio in senescent and fallen leaves or needles declined clearly (Fig. S3), which probably lead to the accumulation of NH_4^+ in those tissues, especially for the Camphor trees (Fig. S1b). Additionally, it was the high pH in fallen leaves or needles that mainly resulted in the high Γ in these aged tissues (Fig. S2). Wang et al. (2013) observed an increase of tissue NH_4^+ concentration, but a decrease of tissue pH thus terminally resulted in the decline of Γ during senescence of the beech leaves. Moreover, it was possible that the decline of chloroplastic glutamine synthetase activity in aged leaves also contributed to the increase of Γ in these tissues (Duan et al. 2012; Mattsson and Schjoerring 1997). Notably, the Γ of the mature Fir needles was generally the highest among the four ages (Fig. 5c), and the high NH_4^+ concentration (Fig. S1c) in the mature parts was the main reason. Small variability of tissue N content in the Fir among different ages was observed (Fig. 6c), implying that the senescence-induced N remobilization within this species was not obvious.

Increases in Γ value may reduce the capacity of forests to act as sinks for the atmospheric NH_3 or even give rise to a release of gaseous NH_3 from plants to the surrounding atmosphere (Wang et al. 2013). The Γ values of the green leaves were about 2600 in an intensively managed grassland (David et al. 2009) and around 2200 in a non-fertilized grassland (Kruit et al. 2007). Wang and Schjoerring (2012) suggested that the senescent grass leaves in all cases had much higher Γ than the green plant tissues (<3000). And in these ranges of Γ value, green leaves are clearly sinks for the atmospheric NH_3 (David et al. 2009; Herrmann et al. 2009). However, the biggest Γ in our study was never exceeded 200, which for the Masson pine needles was less than 2 (Figs. 2c and 5). This relatively low Γ suggested the difficulty of NH_3 emitting from the plants, because the values were too small to build up sufficiently high NH_3 partial pressures in the leaves or needles. With respect to the vast planting areas of the six species at the TSP catchment (Wang et al. 2007), the Masson pine dominant subtropical forest there, was not likely to be a mainly atmospheric NH_3 source. Moreover, Massad et al. (2010) established a clearly exponential relationship between the leaf tissue NH_4^+ and the stomatal NH_3 emission potential (the ratio of apoplastic NH_4^+ to H^+ concentration). According to this correlation, the average of the stomatal compensation point (χ_s) of Masson pine needles in the present study was estimated, i.e., $0.38 \text{ nmol mol}^{-1}$, which was much lower than the seasonal mean concentrations of the atmospheric NH_3 measured at TSP. Additionally, by applying the single-layer resistance model (Massad et al. 2010; Nemitz et al. 2000b; Schrader et al. 2016), the stomatal conductance reported by Zhang (2013) and the leaf area index of Masson pine at the TSP catchment (Wang 2012), we can estimate the NH_3 uptake flux through the stomata (the model details are shown in the

Supporting material). The fluxes were ranging in $0.4\text{--}2.3 \text{ kg NH}_3\text{-N ha}^{-1} \text{ year}^{-1}$, accounting for 1.5–8.5% of $\text{NH}_4^+\text{-N}$ deposition at TSP ($27.2 \text{ kg N ha}^{-1} \text{ year}^{-1}$, yearly average of data from 2012 to 2014, unpublished). These indicate that using the N flux in throughfall only leads to a significant underestimation of total N input in this ecosystem. However, using the values of the stomatal conductance measured by Zhang (2013) leads to some uncertainty on the estimation of the stomatal flux owing to the differences in plant-growing status. Researches should be conducted in future to determine more accurate stomatal conductance at the TSP catchment.

Also, the absorption of gaseous NH_3 through leaf cuticles was quite possible with respect to the much precipitation, the high air humidity (mostly >75%) (Fig. 1), and the high relative water content of the leaves or needles in this study (range: 54.9–86.3% in mature tissues). When the relative air humidity is higher than 70%, gaseous NH_3 may deposit to the leaf surface efficiently (Kruit et al. 2008; van Hove et al. 1989). From these point of views, the present local N budget based on throughfall input only is quite problematic. The gaseous NH_3 uptake through leaf stomata or cuticles contributes largely to the reactive N input at the TSP catchment.

However, the estimation of plants' functioning on the atmospheric NH_3 through Γ values was preliminary and with some degree of uncertainties. Some researchers suggested that the foliage Γ value cannot be used as a tool to predict the potential NH_3 exchange of beech leaves, because the fluctuations of Γ did not match the NH_3 exchange patterns through the chamber method (Wang et al. 2011). However, David et al. (2009) had found a clear relationship between χ_s (estimated from bulk tissue NH_4^+ and bulk pH) and NH_3 fluxes (chamber method), who suggested that the bulk extraction methods were sufficient to identify the main sources and sinks within the grass canopies. Furthermore, a series of researches reported that the bulk tissue NH_4^+ , apoplastic NH_4^+ , and the χ_s were significantly correlated (Hill et al. 2002; Massad et al. 2010; Mattsson and Schjoerring 2002; Mattsson et al. 2009), and positive correlation was also observed between the apoplastic pH and the bulk NH_4^+ concentration (Loubet et al. 2002). Additionally, the dynamic chamber technique itself may overestimate the NH_3 exchange between the plants and the atmosphere, and it was hard to separate the foliage NH_3 emission from those emitted by litter leaves or soils (Hill et al. 2001; Mattsson et al. 2009). Therefore, the foliage Γ have proven to be a promising and convenient indicator to identify the potential source or sink of subtropical forest for the atmospheric NH_3 at the TSP catchment when the χ_s or flux measurements are absent.

Conclusions

Variations of leaf tissue NH_4^+ concentrations and pH among species resulted in very different NH_3 emission potentials. The Fir and Fern leaves or needles had high NH_3 emission potentials,

which for the Pine and Schima were quite low. Higher potential NH_3 emission did not coincide with higher tissue N content in plants. Specifically, the Fir and Pine needles had relatively low N contents, which in the Privet and Fern leaves were higher. Small seasonal variability in NH_3 emission potentials and N pools was observed. Generally, fallen leaves or needles had larger NH_3 emission potentials compared to those at the mature stage. However, the absolute values of NH_3 emission potential of the six species were all too low to build up a sufficiently high NH_3 partial pressure in the leaves or needles. Therefore, the Masson pine dominant subtropical evergreen forest at the TSP catchment functions as a sink for the atmospheric NH_3 , indicating that using the N flux in the throughfall only to stand for the whole reactive N input leads to an underestimation, thereby affects the accuracy of N budget in this ecosystem. This is quite valuable when modeling the leaf-atmosphere NH_3 exchange in forests, and when estimating catchment or regional scales N budget.

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References

- Allen R, Myles L, Heuer MW (2011) Ambient ammonia in terrestrial ecosystems: a comparative study in the Tennessee Valley, USA. *Sci Total Environ* 409:2768–2772. doi:10.1016/j.scitotenv.2011.04.017
- Beusen AHW, Bouwman AF, Heuberger PSC, Van Drecht G, Van Der Hoek KW (2008) Bottom-up uncertainty estimates of global ammonia emissions from global agricultural production systems. *Atmos Environ* 42:6067–6077. doi:10.1016/j.atmosenv.2008.03.044
- Chen X, Mulder J (2007) Atmospheric deposition of nitrogen at five subtropical forested sites in South China. *Sci Total Environ* 378:317–330. doi:10.1016/j.scitotenv.2007.02.028
- David M, Loubet B, Cellier P, Mattsson M, Schjoerring JK, Nemitz E, Roche R, Riedo M, Sutton MA (2009) Ammonia sources and sinks in an intensively managed grassland canopy. *Biogeosciences* 6:1903–1915. doi:10.5194/bg-6-1903-2009
- Dong W, Xing J, Wang S (2010): Temporal and spatial distribution of anthropogenic ammonia emissions in China: 1994–2006. *Chi J Environ Sci* 31, 1457–1463 (in Chinese with English abstract).doi:10.13227/j.hjkk.2010.07.008
- Duan W, Yang T, Dai Y, Li D, Zhang X, Liu H, Li N, Wang CG (2012) Stomatal NH_3 compensation point and its metabolic regulation in senescence phenotypes of *Nicotiana tabacum*. *Biol Plantarum* 56:771–774. doi:10.1007/s10535-012-0141-z
- Flechard CR, Massad RS, Loubet B, Personne E, Simpson D, Bash JO, Cooter EJ, Nemitz E, Sutton MA (2013) Advances in understanding, models and parameterizations of biosphere-atmosphere ammonia exchange. *Biogeosciences* 10:5183–5225. doi:10.5194/bg-10-5183-2013
- Geßler A, Rienks M, Rennenberg H (2000) NH_3 and NO_2 fluxes between beech trees and the atmosphere—correlation with climatic and physiological parameters. *New Phytol* 147:539–560. doi:10.1046/j.1469-8137.2000.00712.x
- Hansen K, Sorensen LL, Hertel O, Geels C, Skjoth CA, Jensen B, Boegh E (2013) Ammonia emissions from deciduous forest after leaf fall. *Biogeosciences* 10:4577–4589. doi:10.5194/bg-10-4577-2013
- Herrmann B, Mattsson M, Jones SK, Cellier P, Milford C, Sutton MA, Schjoerring JK, Neftel A (2009) Vertical structure and diurnal variability of ammonia exchange potential within an intensively managed grass canopy. *Biogeosciences* 6:15–23. doi:10.5194/bg-6-15-2009
- Hill PW, Raven JA, Loubet B, Fowler D, Sutton MA (2001) Comparison of gas exchange and bioassay determinations of the ammonia compensation point in *Luzula sylvatica* (Huds.) gaud. *Plant Physiol* 125:476–487. doi:10.1104/pp.125.1.476
- Hill PW, Raven JA, Sutton MA (2002) Leaf age-related differences in apoplastic NH_4^+ concentration, pH and the NH_3 compensation point for a wild perennial. *J Exp Bot* 53:277–286. doi:10.1093/jexbot/53.3.277
- van Hove LWA, Adema EH, Vredenberg WJ, Pieters GA (1989) A study of the adsorption of NH_3 and SO_2 on leaf surfaces. *Atmos Environ* 23:1479–1486. doi:10.1016/0004-6981(89)90407-1
- van Hove LWA, Heeres P, Bossen ME (2002) The annual variation in stomatal ammonia compensation point of ryegrass (*Lolium perenne* L.) leaves in an intensively managed grassland. *Atmos Environ* 36:2965–2977. doi:10.1016/S1352-2310(02)00242-X
- Huang X, Song Y, Li MM, Li JF, Huo Q, Cai XH, Zhu T, Hu M, Zhang HS (2012) A high-resolution ammonia emission inventory in China. *Global Biogeochem Cy* 26:GB1030. doi:10.1029/2011gb004161
- Jiang C, Zhang X (2008) N isotopes and N cycle in the TieShanPing subtropical forest ecosystem, southwestern China. *Environ Monit Assess* 154:301–308. doi:10.1007/s10661-008-0398-z
- Kruit RJW, van Pul WAJ, Otjes RP, Hofschreuder P, Jacobs AFG, Holtslag AAM (2007) Ammonia fluxes and derived canopy compensation points over non-fertilized agricultural grassland in the Netherlands using the new gradient ammonia - high accuracy - monitor (GRAHAM). *Atmos Environ* 41:1275–1287. doi:10.1016/j.atmosenv.2006.09.039
- Kruit RJW, Jacob AFG, Holtslaga AAM (2008) Measurements and estimates of leaf wetness over agricultural grassland for dry deposition modeling of trace gases. *Atmos Environ* 42:5304–5316. doi:10.1016/j.atmosenv.2008.02.061
- Krupa SV (2003) Effects of atmospheric ammonia (NH_3) on terrestrial vegetation: a review. *Environ Pollut* 124:179–221. doi:10.1016/S0269-7491(02)00434-7
- Li Z, Wang Y, Liu Y, Guo H, Li T, Li Z, Shi G (2014) Long-term effects of liming on health and growth of a masson pine stand damaged by soil acidification in Chongqing, China. *PLoS One* 9:e94230. doi:10.1371/journal.pone.0094230
- Loubet B, Milford C, Hill PW, Sim Tang Y, Cellier P, Sutton MA (2002) Seasonal variability of apoplastic NH_4^+ and pH in an intensively managed grassland. *Plant Soil* 238:97–110. doi:10.1023/a:1014208926195
- Lun X, Zhang X, Mu Y, Anpu N, Anpu JG (2003) Size fractionated speciation of sulfate and nitrate in airborne particulates in Beijing, China. *Atmos Environ* 37:2581–2588. doi:10.1016/s1352-2310(03)00220-6
- Masclaux C, Valadier MH, Brugiére N, Morot-Gaudry JF, Hirel B (2000) Characterization of the sink/source transition in tobacco (*Nicotiana tabacum* L.) shoots in relation to nitrogen management and leaf senescence. *Planta* 211:510–518. doi:10.1007/s004250000310
- Massad RS, Nemitz E, Sutton MA (2010) Review and parameterisation of bi-directional ammonia exchange between vegetation and the atmosphere. *Atmos Chem Phys* 10:10359–10386. doi:10.5194/acp-10-10359-2010
- Mattsson M, Schjoerring JK (1996) Ammonia emission from young barley plants: influence of N source, light/dark cycles

- and inhibition of glutamine synthetase. *J Exp Bot* 47:477–484. doi:10.1093/jxb/47.4.477
- Mattsson M, Schjoerring J (1997) Ammonia exchange between plants and the atmosphere: Effects of ammonium supply to the roots, dark-induced senescence and reduced GS activity. In: Ando T et al. (Editors), *Plant Nutrition for Sustainable Food Production and Environment. Developments in Plant and Soil Sciences*. Springer Netherlands, pp. 827–831
- Mattsson M, Schjoerring JK (2002) Dynamic and steady-state responses of inorganic nitrogen pools and NH₃ exchange in leaves of *Lolium perenne* and *Bromus erectus* to changes in root nitrogen supply. *Plant Physiol* 128:742–750. doi:10.1104/pp.010602
- Mattsson M, Schjoerring JK (2003) Senescence-induced changes in apoplastic and bulk tissue ammonia concentrations of ryegrass leaves. *New Phytol* 160:489–499. doi:10.1046/j.1469-8137.2003.00902.x
- Mattsson M, Herrmann B, Jones S, Neftel A, Sutton MA, Schjoerring JK (2009) Contribution of different grass species to plant-atmosphere ammonia exchange in intensively managed grassland. *Biogeosciences* 6:59–66. doi:10.5194/bg-6-59-2009
- Neirynek J, Ceulemans R (2008) Bidirectional ammonia exchange above a mixed coniferous forest. *Environ Pollut* 154:424–438. doi:10.1016/j.envpol.2007.11.030
- Neirynek J, Kowalski AS, Carrara A, Ceulemans R (2005) Driving forces for ammonia fluxes over mixed forest subjected to high deposition loads. *Atmos Environ* 39:5013–5024. doi:10.1016/j.atmosenv.2005.05.027
- Nemitz E, Sutton MA, Gut A, José RS, Husted S, Schjoerring JK (2000a) Sources and sinks of ammonia within an oilseed rape canopy. *Agric For Meteorol* 105:385–404. doi:10.1016/S0168-1923(00)00205-7
- Nemitz E, Sutton MA, Schjoerring JK, Husted S, Wyers GP (2000b) Resistance modelling of ammonia exchange over oilseed rape Eiko Nemitz. *Agric For Meteorol* 105:405–425. doi:10.1016/S0168-1923(00)00206-9
- Nie W, Wang T, Gao X, Pathak RK, Wang X, Gao R, Zhang Q, Yang L, Wang W (2010) Comparison among filter-based, impactor-based and continuous techniques for measuring atmospheric fine sulfate and nitrate. *Atmos Environ* 44:4396–4403. doi:10.1016/j.atmosenv.2010.07.047
- Rennenberg H, Kreutzer K, Papen H, Weber P (1998) Consequences of high loads of nitrogen for spruce (*Picea abies*) and beech (*Fagus sylvatica*) forests. *New Phytol* 139:71–86. doi:10.1046/j.1469-8137.1998.00181.x
- Schjoerring JK, Mattsson M (2001) Quantification of ammonia exchange between agricultural cropland and the atmosphere: measurements over two complete growth cycles of oilseed rape, wheat, barley and pea. *Plant Soil* 228:105–115. doi:10.1023/A:1004851001342
- Schjoerring JK, Husted S, Mattsson M (1998) Physiological parameters controlling plant-atmosphere ammonia exchange. *Atmos Environ* 32:491–498. doi:10.1016/S1352-2310(97)00006-X
- Schrader F, Brummer C, Flechard CR, Kruit RJW, van Zanten MC, Zoll U, Hensen A, Erisman JW (2016) Non-stomatal exchange in ammonia dry deposition models: comparison of two state-of-the-art approaches. *Atmos Chem Phys* 16:13417–13430. doi:10.5194/acp-16-13417-2016
- Stamenkovic LJ, Antanasijevic DZ, Ristic MD, Peric-Grujic AA, Pocajt VV (2015) Modeling of ammonia emission in the USA and EU countries using an artificial neural network approach. *Environ Sci Pollut R* 22:18849–18858. doi:10.1007/s11356-015-5075-5
- Sutton MA, Gut A, San José R, Husted S, Schjoerring JK (2001) Biosphere-atmosphere interactions of ammonia with grasslands: experimental strategy and results from a new European initiative. *Plant Soil* 228:131–145. doi:10.1023/a:1004822100016
- Sutton MA, Erisman JW, Dentener F, Moller D (2008) Ammonia in the environment: from ancient times to the present. *Environ Pollut* 156:583–604. doi:10.1016/j.envpol.2008.03.013
- Wang Y (2012) Characteristics of stand structure and hydrological function of damaged Masson pine forests in the acid rain region of Chongqing. Dissertation, Chinese Academy of Forest, Beijing
- Wang L, Schjoerring JK (2012) Seasonal variation in nitrogen pools and ¹⁵N/¹³C natural abundances in different tissues of grassland plants. *Biogeosciences* 9:1583–1595. doi:10.5194/bg-9-1583-2012
- Wang Y, Solberg S, Yu P, Myking T, Vogt RD, Du S (2007) Assessments of tree crown condition of two Masson pine forests in the acid rain region in South China. *For Ecol Manag* 242:530–540. doi:10.1016/j.foreco.2007.01.065
- Wang L, Xu Y, Schjoerring JK (2011) Seasonal variation in ammonia compensation point and nitrogen pools in beech leaves (*Fagus sylvatica*). *Plant Soil* 343:51–66. doi:10.1007/s11104-010-0693-7
- Wang L, Ibrom A, Korhonen JFJ, Arnoud Frumau KF, Wu J, Pihlatie M, Schjoerring JK (2013) Interactions between leaf nitrogen status and longevity in relation to N cycling in three contrasting European forest canopies. *Biogeosciences* 10:999–1011. doi:10.5194/bg-10-999-2013
- WRB (2006) World reference base for soil resources. FAO, Rome, 3 b. pp
- Zhang X (2013) Photosynthetic Physiology for *Pinus massoniana* in Jinyun Mountain. Dissertation, Beijing Forestry University, Beijing
- Zhang L, Wright LP, Asman WAH (2010) Bi-directional air-surface exchange of atmospheric ammonia: a review of measurements and a development of a big-leaf model for applications in regional-scale air-quality models. *J Geophys Res-Atmos* 115:D20310. doi:10.1029/2009jd013589
- Zhu J, Mulder J, Solheimslid SO, Dörsch P (2013) Functional traits of denitrification in a subtropical forest catchment in China with high atogenic N deposition. *Soil Biol Biochem* 57:577–586. doi:10.1016/j.soilbio.2012.09.017
- Zou Q (2000) *Plant Physiology Experiment Instruction*. China Agriculture Press (in Chinese), Beijing