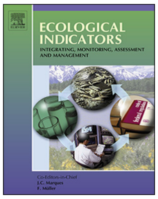
Ecological Indicators 93 (2018) 813–821

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Original Articles

Variations in free amino acid concentrations in mosses and different parts of *Cinnamomum camphora* along an urban-to-rural gradient

Yu Xu[a,b], Huayun Xiao[a,*], Hui Guan[a], Yanli Wang[c], Chaojun Long[a,b], Jing Zhao[a,b]

[a] *State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, No. 99, Linchengxi Road, Guiyang 550081, China*
[b] *University of Chinese Academy of Sciences, Beijing 100049, China*
[c] *Chinese Academy for Environmental Planning, Beijing 100012, China*

ARTICLE INFO

Keywords:
Plant amino acids
Bioindication
Amino acid cycle
Nitrogen deposition

ABSTRACT

Vegetation growing in urban ecosystems is frequently exposed to an environment with high atmospheric nitrogen (N) pollution. We systematically investigated the free amino acid concentrations in moss samples and *Cinnamomum camphora* leaf (new, middle-aged and old leaves), branch phloem, trunk phloem, and bark samples from field sites (Guiyang City, SW China) with different N deposition levels. The responses of the free amino acids to N deposition were analysed in the abovementioned plant tissues to determine whether plant free amino acids could act as biomarkers of the external N supply; moreover, differences in the N metabolism of these tissues under varying N deposition conditions were revealed by the compositions and sizes of their free amino acid pools. In particular, we reported the significant accumulation of arginine with increased N deposition in bark samples (while the arginine concentrations in the branch phloem, trunk phloem, and leaves remained low), which may indicate a long-term or historic external environment with a high N availability; additionally, the noticeable dominance and fluctuation of γ-aminobutyric acid in response to varied N deposition levels occurred in both the branch phloem and the trunk phloem, suggesting that the γ-aminobutyric acid transported in the phloem may be used as an important signal reflecting increases in the atmospheric N input. We conclude that the free amino acid concentrations in moss and camphor leaf, phloem and bark tissues are more sensitive to N deposition compared to their N concentrations and that tissue glutamine/glutamate and arginine/γ-aminobutyric acid ratios may serve as better biomarkers reflecting the tissue N accumulation status associated with increased N deposition. Therefore, free amino acid concentration analyses of different plant parts may provide a means to gain a more in-depth understanding of the impacts of atmospheric N pollution on plant physiology and N cycles.

1. Introduction

High anthropogenic emissions of reactive N have detrimental effects on the atmospheric quality and the health of terrestrial and aquatic ecosystems (Menon et al., 2007; Clark and Tilman 2008; Hastings et al., 2013; van Zanten et al., 2017). In contrast, N compounds from anthropogenic pathways also provide a significant input of available N via wet and dry deposition to soil and plants, which has received particular attention due to its potentially positive effects on plant growth (Townsend et al., 1996; Aber and Driscoll, 1997; Norby, 1998; Bauer et al., 2004). It is well known that the N demand of vascular plants is partly met by the root uptake of ammonium and nitrate with associated microbial processes, soil properties and natural or artificial factors (Magill et al., 2000; Mo et al., 2008; Cernusak et al., 2009; Xuan et al., 2017). If the available soil N is insufficient, the remobilization of N from internal plant resources can supply most N nutrition required for the development of new tissues (Arthur et al., 1998; Cherbuy et al., 2001; Millard and Grelet, 2010). Particularly in the early spring, low soil temperature leads to a lack of N nutrition for plants (Millard et al., 2006; Li et al., 2016). Additionally, N deposited from the atmosphere (mainly NH_x-N, NO_x-N and organic N) can be substantially absorbed or absorbed by aboveground plant parts in gaseous, dissolved, or particulate forms (Schulz et al., 2001; Lockwood et al., 2008; Padgett et al., 2009; Varela et al., 2016; Xu et al., 2017), causing some terrestrial vegetation to be partly independent of the soil N supply. For example, a study conducted by Ammann et al. (1999) found that oxidized N compounds represented approximately 25% of the N nutrition contribution to potted Norway spruce needles after the plants were placed near a highway for 4 months. Increases in the needle N concentration and biomass were also observed in Scots pine exposed to an

---

[*] Corresponding author at: Institute of Geochemistry, Chinese Academy of Sciences, Lincheng West Road (Jinyang) 99, Guiyang, Guizhou Province 550081, China.
E-mail address: xiaohuayun@vip.skleg.cn (H. Xiao).

https://doi.org/10.1016/j.ecolind.2018.04.030
Received 18 December 2017; Received in revised form 3 April 2018; Accepted 12 April 2018

environment with high gaseous NH_3 levels, corroborating that atmospheric NH_3 inputs can be used as exogenous N fertilizer to maintain plant growth (Pérez-Soba and Van der Eerden, 1993).

Soil N (nitrate, ammonium and some organic N compounds) assimilated by plant roots or remobilized N from storage tissues is transported to tissues with N requirements partly or even largely in the form of amino acids through the xylem and/or phloem (Caputo and Barneix, 1997; Gourieroux et al., 2016; Tegeder and Hammes, 2018). Xylem transport is primarily governed by the transpiration stream in plants, but phloem transport is largely dependent on the relative status of the tissue N requirements, with transport directed from N sources to N sinks; moreover, N nutrients, such as free amino acids, are interchangeable between two transport systems (Arthur et al., 1998). In addition, according to the results of isotope labelling experiments, the atmospheric N compounds absorbed by plant leaves have long been known to be first incorporated into free amino acids (Nussbaum et al., 1993; Weber et al., 1995; Padgett et al., 2009). Thus, the pools and fluxes of free amino acid-N in different parts of plants play a significant part in protein synthesis and tissue N transport.

Based on this critical role of amino acids in plant N metabolism, further studies have found that plants are impacted by and show changes in the composition and size of their amino acid pools in response to different environmental stresses (e.g., water, mineral nutrient, heavy metal, N addition, pest damage, pathogens, temperature and salt stress); during these studies, arginine, γ-aminobutyric acid, asparagine, glutamine, glutamate, aspartate and proline have attracted close attention because of their significant accumulations (Chiozza et al., 2010; Liu et al., 2011; Garde-Cerdán et al., 2014; Hammad and Ali, 2014; Pavlíková et al., 2014; Postles et al., 2016). Such changes can thus be used as signals for indicating varied environmental conditions and the influences of environmental stress on plant N metabolism. Although previous studies have provided a large amount of information with regard to the potential effects of environmental stresses on the free amino acid concentrations of plant tissues, most of the experiments were performed in laboratories by simulating various environmental stresses or in natural habitats by the anthropogenic addition of the target substance (particularly simulated N deposition via fertilization). Furthermore, these earlier studies mainly focused on the mean concentrations of free amino acids in whole leaves; however, the determination of whole leaf results fails to consider differences in the free amino acid concentrations of leaves of different ages. In comparison, while a few studies investigating the responses of free amino acids in vascular plant leaves to changes in atmospheric N deposition (Power and Collins, 2010), studies on N deposition-induced variations in free amino acid concentrations in different plant parts (e.g., new, mature and old leaves, branch phloem and trunk phloem) are still limited, especially in the actual field environment. To date, several studies have fully evaluated the correlation between the chemical compositions of bark (outermost layer of the tree trunk) and atmospheric pollutants in an effort to find a proxy for the atmospheric pollution status (Schulz et al., 1997; Saarela et al., 2005; Kwak et al., 2009; Boltersdorf et al., 2014). These chemical indicators in the bark mainly included the $\delta^{15}N$, $\delta^{13}C$, N concentration, NO_3^- and NH_4^+ concentrations, and metal element concentrations. However, variations in the free amino acid concentrations of bark from sites with different atmospheric N pollution conditions have not been studied in detail, and their ability to record environmental N changes needs to be further confirmed.

Increasingly, analyses determining the positive and negative environmental effects of N pollution have focused on how N deposition impacts plant tissue nutrient N budgets and tissue N chemistry (Koranda et al., 2006; Padgett et al., 2009). To determine how the absorption and transport of N responds to the requirements of N-consuming tissues under different atmospheric N deposition levels, a signal for identifying the N status of plant tissues is needed; this signal can also be used as an indicator of increases in the atmospheric N input (Huhn and Schulz, 1996; Pitcairn et al., 2003). It has been suggested that the transport of amino compounds from the shoot to the roots through the phloem can reflect the plant N status (Weber et al., 1998). Such a signal associated with amino acid cycling between the N supply tissues and the N demand tissues also has critical roles in the regulation of the external N absorption by plants, such that the accumulated amino acid pools of these processes indicate both the external N supply and the internal N status of plants (Muller et al., 1996; Arthur et al., 1998). This proposal has been supported by experiments about the influence of anthropogenic N additions on the metabolism of N in conifers (Nordin et al., 2001). However, we need to extend this analysis to examine the consequences of high atmospheric N deposition (rather than N addition) for amino acid allocation and cycle in plants.

In this study, we analysed the variations in the free amino acid pools of mosses and different parts of camphor trees (new, middle-aged and old leaves, branch phloem, trunk phloem and the outermost bark) from field sites with different atmospheric N inputs. *Cinnamomum camphora* was selected for the study because it is widely distributed in Guiyang City as a main species used in urban greening. It is known that bryophytes primarily rely on atmospheric N to maintain their N requirements (Wilson et al., 2009; Skudnik et al., 2015). Based on this unique physiological characteristic of bryophytes, they are regarded as an excellent contrastive material to reveal the variability in the free amino acid pools in different parts of the camphor trees grown in the different field sites. The principal objectives of the present study were to determine which amino compounds are important indicators of the N nutrition status of the investigated plants and whether sensitive parameters exist in plants exposed to high atmospheric N inputs; furthermore, if these parameters do exist, what are the "dose-effect" relationships of N influence?

2. Materials and methods

2.1. Site characteristic

This study was conducted in Guiyang City (SW China) in a wide karst valley basin. The dense population and transportation network and rapidly developing industrial production and agricultural activities have made the city suffer serious environmental pollution (Tian et al., 2013; Liu et al., 2017). Sampling sites were chosen in the urban center (0–6 km), semi-urban areas (6–12 km area from the urban center), suburban areas (12–24 km area from the urban center) and rural areas (more than 24 km areas from the urban center) based on their different environmental characteristics (Fig. 1). The average total N deposition levels in the aforementioned areas were 29.21 ± 6.17, 21.98 ± 8.34, 11.64 ± 3.78, and 16.78 ± 4.32 $kg\,N\,ha^{-1}\,yr^{-1}$, respectively (Liu et al., 2009). This N deposition pattern, which decreases from the urban center to the suburban areas, is consistent with observation of atmospheric NO_2 concentrations, with average levels of 39.1 $\mu g\,m^{-3}$ in the urban center and 18.6 $\mu g\,m^{-3}$ in the suburban areas (Xu et al., 2018). The soil type in all the study areas was the same, characterized by acidic yellow soil with a low base saturation and high aluminium concentrations (Bohan et al., 1997). In addition, the average N concentrations and the average $\delta^{15}N$ values of the soil samples collected from the rooting zone at a depth of 0–10 cm in each area were not significantly different (data not shown). All sampling sites were at least 60 m away from the nearest pollutant sources and main roads.

2.2. Collection and treatment of samples

Sampling was carried out in June 2016 in either sunny or cloudy weather. New, middle-aged and old leaf, branch phloem, trunk phloem and outermost trunk (bark) samples from camphor trees approximately 8 m in height and approximately 15 years old were collected at 60 sites across the urban to rural gradient in Guiyang City (Fig. 1). Approximately 4–6 g of new current-year leaves (new, verdant leaves at the shoot apex with surface areas less than 10 cm^2), mature current-year