biologia plantarum
an international journal for experimental botany

BIOLOGIA PLANTARUM (2026) 70: 18-26 DOI: 10.32615/bp.2026.003

Composition of organic acids secreted by alpine shrub roots and its influencing factors

Chen-Kang XI[1,2], Ling ZHU[3], Naveed AHMED[4], and Yong-Heng GAO[1,*]

[1] Chengdu Institute of Biology, Chinese Academy of Science, Chengdu 610213, P.R. China
[2] College of Life Sciences, University of Chinese Academy of Sciences, Beijing 100049, P.R. China
[3] Institute of Mountain in Hazards and Environment, Chinese Academy of Sciences, Chengdu 610213, P.R. China
[4] The National Key Laboratory of Water Disaster Prevention, College of Hydrology and Water Resources, Hohai University, Nanjing 210098, P.R. China

*Corresponding author: E-mail: gaoyh@cib.cas.cn

Abstract

Background and aims: There appears to be a strong correlation between the rapid proliferation of shrubs in the alpine grassland of the Qinghai-Tibet Plateau and the root exudates of these plants. The dynamics of root exudates during shrub development, however, have been the subject of few investigations.
Methods: This work examined *Lonicera thibetica*, a plant found on the eastern edge of the Qinghai-Tibet Plateau. It focused on the root systems of 5-, 10-, 15-, and 20-year-old *L. thibetica* plants and how their primary organic acid components changed with the seasons and other circumstances.
Results: The findings revealed that the primary components of acid secretion in the roots of *L. thibetica* were oxalic acid, lactic acid, and tartaric acid; of these, oxalic acid made up over 50% of the total organic acid content. The levels of these organic acids often dropped as shrubs got older, and their seasonal dynamics exhibited a parabolic shift pattern, typically peaking during the robust development stage. According to regression analysis, soil moisture was the primary determinant of the concentration of organic acids produced by alpine shrub roots, suggesting that soil moisture played a crucial role in the secretion process.
Conclusions: The physiological dynamics of roots and the mechanisms that regulate them throughout the expansion of alpine shrubs can be better understood thanks to this work.

Keywords: organic acid, seasonal dynamics, shrubification, soil moisture.

Introduction

Due to climatic warming and human activity, 10-20% of the world's grasslands have been converted to shrubland (Wang et al., 2018). Shrubification is happening quickly in grassland ecosystems at high altitudes and in high latitudes as well (Wang et al., 2019). As the third pole of the world, about 10% of the alpine meadows in the Qinghai-Tibet Plateau have been scrubbed in the past 30 years, and it is expected to continue to expand in the future (Zhao et al., 2021). In the process of alpine meadow shrubification, its root exudates play an important role in changing the soil ecological environment of the invaded area and promoting the adaptive growth and reproduction of the expanding shrubs (Cai et al., 2024). Root exudates are various organic substances secreted by plant roots to the rhizosphere, accounting for 5 to 21% of plant photosynthetic products (Shahzad et al., 2015). These secretions are complex and diverse, and play an important role in plant growth, soil

Received 1 July 2025, *last revision* 2 December 2025, *accepted* 5 February 2026.
Abbreviations: AST - average soil temperature; ASWC - average soil moisture content in the growing season; ST - soil temperature; SWC - soil moisture content.
Acknowledgements: This research was supported by the National Natural Science Foundation of China (32371649), and the Key Research and Development Program of the Sichuan Province of China (2024YFTX0015).
Data availability: Data will be made available on request.
Conflict of interest: The authors declare that they have no conflict of interest.

quality improvement, and ecosystem balance maintenance. Organic acids are an important component of root exudates (Adeleke et al., 2017; Macias-Benitez et al., 2020), whose average content can reach 10 - 30% of the total root exudates (Van Hees and Lundström, 2000). Organic acids are not only a key medium for information exchange and energy transfer between plants and soil, but also play an important role in regulating nutrient acquisition, shaping rhizosphere microbial community structure and alleviating environmental stress (Gómez-Romero et al., 2024). However, the specific mechanism of organic acids in root microecosystems and their interaction with environmental factors are still unclear.

The concentration of organic acids secreted by plant roots is affected by many factors (Chen et al., 2023). According to the theory of energy limitation, with the increase of tree age, the carbon input of plants to underground biomass decreases, resulting in a large difference in root exudation during their lifetime (Wasyliw and Karst, 2020). During the sapling period, photosynthesis is strong and root exudation is high, which promotes its rapid growth (Gargallo-Garriga et al., 2018; Weinhold et al., 2022). In addition, the concentration of root exudates is also regulated by environmental factors such as temperature, precipitation, and light (Meier et al., 2013). For example, a study of root exudates in Mediterranean forests showed that the concentration of root exudates in trees would change significantly during the year due to changes in environmental factors such as soil water content and soil temperature, resulting in significant seasonal differences in root exudates concentration (Jakoby et al., 2020). However, the existing research mainly focuses on the root exudates of trees in low altitude areas (Chen et al., 2023; Gao et al., 2024), but there are few reports on the study of root exudates of alpine shrubs. Due to the unique environmental conditions in the alpine region, factors such as low temperature, strong radiation and short growing season may have a significant impact on the composition and function of root exudates. Studying the dynamic changes of root acid secretion of alpine shrubs is of great significance for revealing the physiological and ecological mechanism of plant adaptation to alpine environment.

L. thibetica belongs to the family Caprifoliaceae, genus *Lonicera*. It is one of the main shrub community types in the Qinghai-Tibet Plateau. It is mainly distributed in the slopes, forest edges and river valleys at an altitude of 3 400 - 4 000 m, with a clear expansion trend. *L. thibetica* is a pioneer species in alpine meadow shrubification on the Qinghai-Tibet Plateau, capable of colonizing shallow soils (Cao et al., 2022). It is a weak calcicole, preferring slightly alkaline soils and tolerating neutral soils, with moderate drought tolerance, sensitivity to acidic soils, and vulnerability to root rot under prolonged waterlogging (Adeleke et al., 2017; Yang et al., 2024). In this study, *L. thibetica* shrubs in the eastern margin of the Qinghai-Tibet Plateau were used as the research object to explore the changes of the main organic acid concentrations secreted by *L. thibetica* shrubs of different ages during the growing season (June - September) and their relationship with soil moisture and temperature, aiming to provide a theoretical basis for understanding the process and mechanism of shrub expansion in alpine grasslands.

Materials and methods

Study sites: In the eastern part of the Qinghai-Tibet Plateau, the research area may be found in Qiongxi Town, Hongyuan County, Aba Prefecture, Sichuan Province. It is situated at an average elevation of approximately 3 500 m and is located at 33° 03' N, 102° 36' E. In the county, the yearly average temperature is approximately 1.1℃, and the annual average precipitation is from 600 to 800 mm, with the majority of precipitation falling between the months of June and September. Alpine meadow soil is the type of soil that may be found in this region, while alpine meadow vegetation is the predominant form of flora. The species that predominate include *Kobresia setchwanensis* and *Elymus nutans*, with *Gueldenstaedtia diversifolia*, *Aster alpina*, and *Anemone trullifolia* following closely after (Xie and Gao, 2015). According to Cao et al. (2022), the shrubs that are most commonly found in this region include *L. thibetica*, *Salix oritrepha*, and *Dasiphora fruticosa* species. The tested *L. thibetica* grows on well-drained slopes (5 - 10°; non-runoff-convergent areas) in naturally successional communities, consistent with alpine shrubs' habitat preference on the Qinghai-Tibet Plateau. The study meadow has a history of grazing (proven to drive *Lonicera* community succession) and is currently under grazing exclusion, with an exploitable 60 - 80 cm deep alpine meadow soil (sandy loam, no hypoxic layer) – its organic matter content ranges from 8.2 - 10.5% (0 - 20 cm) to 1.9 - 2.7% (40 - 60 cm) (Lian et al., 2024). July soil solution pH was neutral (7.0 - 7.3 for 0 - 20 cm; 7.2 - 7.5 for deeper layers), with 0 - 20 cm percolation water having higher Ca^{2+}, Fe^{2+}, Mn^{2+}, and Al^{3+} concentrations than deeper layers.

Collection and determination of root exudates: In this study, *L. thibetica* bushes from Tibet were used as research objects for periods of 5, 10, 15, and 20 years. Table 1 displays, for various ages, the plant height and trunk circumference of *L. thibetica* bushes in Tibet. A monthly sample of the organic acids released by *L. thibetica* roots was taken using an *in-situ* collecting method from June to September of 2020 (He et al., 2017). For the digging process, only healthy roots measuring 20 - 30 cm in length and less than 2 mm in diameter were chosen. The roots were meticulously cleaned with distilled water and then irrigated using plastic bags when they had been unearthed to their full depth. Following the cleaning process, the roots were carefully placed into a sterile medical injection syringe and the needles were carefully covered with a nylon net. Sterile glass beads were added to simulate soil resistance, and then 40 ml carbon-free nutrient solution (main components: 12 mmol/L KH_2PO_4, 0.5 mmol/L NH_4NO_3, 0.2 mmol/L K_2SO_4, 0.3 mmol/L $CaCl_2$, 0.2 mmol/L $MgSO_4$ (Chen et al., 2023)) was injected

Table 1. Plant height and trunk circumference of *Lonicera thibetica* shrubs of different ages. Means ± SEs, $n = 4$.

Age [year]	Plant height [cm]	Trunk circumference [cm]
5	29.83 ± 0.18	2.10 ± 1.14
10	46.33 ± 0.48	3.18 ± 1.94
15	76.67 ± 0.11	3.93 ± 1.96
20	91.67 ± 0.25	4.69 ± 2.39

to maintain root activity. At the same time, glass wool (soaked in 2 mol/L HCl for 24 h) was placed at the bottom, and the needle cylinder was wrapped with aluminum foil paper. After the *in-situ* collection device was buried in the soil for two days, the nutrient solution was extracted with a portable vacuum pump and washed three times to ensure no residue. After rinsing, 40 mL of nutrient solution was injected, and extraction sampling was performed 3 days later. The root exudates of *L. thibetica* of the same age were repeatedly sampled for three times, and the qualitative and quantitative analysis of organic acids was carried out by *Agilent 1 260* high performance liquid chromatograph (HPLC, *Agilent Technologies*, Santa Clara, CA, USA). During the 3 days pre- and post-sample collection (June - September), no rainfall or fog was recorded at the study site; daily photosynthetically active radiation (PAR) averaged 1 400 – 1 600 μmol $m^{-2}\ s^{-1}$, and air temperature ranged from 12 to 18°C. Two key preservation measures were implemented: 0.1% (v/v) sodium azide (a microbial inhibitor) was immediately added post-collection to suppress bacterial and fungal activity, and all samples were stored at 4°C and analyzed *via* HPLC within 6 h of sampling. This approach aligns with standard root exudate preservation protocols.

Determination of soil moisture content and soil temperature: At the same time of collecting root exudates, the debris of the soil surface layer within 20 cm of the measured shrub patch was removed, and the soil moisture and temperature at 20 cm depth were measured by portable water and soil temperature detector, with 3 replicates each time. We used a capacitive probe (*Model TDR300*, *Spectrum Technologies*, Aurora, IL, USA) for measurements. To ensure representativeness, we set 3 sampling points per plot, all targeting the 0 - 20 cm soil layer (consistent with root exudate sampling depth).

Statistical analysis: *SPSS Statistics 22.0* (*SPSS Inc.*, Chicago, IL, USA) was used for statistical analysis of the data. The data were tested for normality and homogeneity of variance to ensure that the prerequisites for analysis of variance were met. One-way analysis of variance (One-way *ANOVA*) was used to compare the seasonal differences in soil moisture content, soil temperature and root exudates of *L. thibetica* shrubs at different ages, and multiple comparisons were performed by least significant difference (LSD) method to determine the significance of the differences between groups ($P < 0.05$). Linear regression analysis was used to explore the correlation between organic acid concentration and soil moisture content and temperature. All graphics in this article are drawn using *Origin 2022* (*OriginLab Corporation*, Northampton, MA, USA).

Results

Age variation and seasonal dynamics of soil moisture content and temperature in shrub patches of *L. thibetica*: In shrub patches of *L. thibetica* in Tibet, there was a notable variation in soil moisture content across several age groups (Fig. 1*A*, $P < 0.05$). In terms of average soil moisture content, the shrub patches that were 10 years old had the highest level at 29.47%, while the patches that were 20 years old had the lowest level at 19.42%. Soil moisture content followed a seasonal peak-and-fall pattern, with August being the typical high point and September being the low point (Fig. 1*B*). The soil temperature of the shrub patches varied between 15.25 and 16.27°C throughout the growing season and exhibited a single-peak pattern ($P > 0.05$, Fig. 1*C*,*D*).

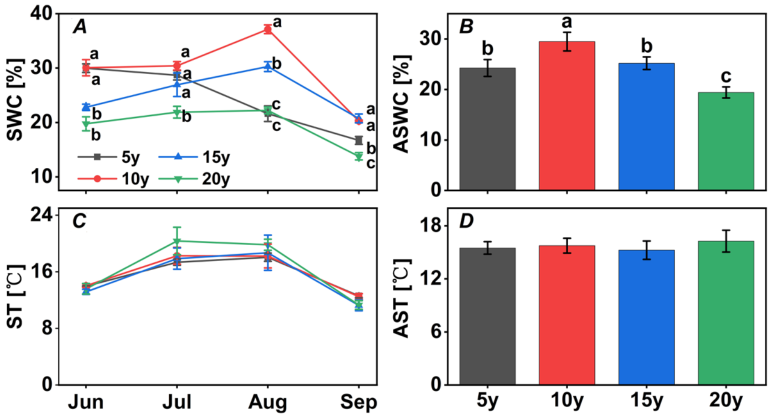

Fig. 1. Age variation and seasonal dynamics of soil water content (*A*, *B*) and temperature (*C*, *D*) in shrub patches of *Lonicera thibetica* in Tibet. SWC - soil moisture content, ASWC - average soil moisture content in the growing season, ST - soil temperature, AST - average soil temperature in the growing season. Means ± SEs ($n = 4$). Points and bars bearing *lowercase letters* ($P < 0.05$) differ significantly between different ages as determined by LSD test, and the *unmarked points and bars* represent no difference; 5y, 10y, 15y, 20y represent 5 years, 10 years, 15 years, 20 years of *L. thibetica* shrubs.

Composition of organic acids secreted by roots of *L. thibetica* shrub at different ages: Among the 10 organic acids secreted by the roots of *L. thibetica* in Tibet (Fig. 2), the concentration of oxalic acid was the highest, accounting for more than 50% of the total organic acid concentration, and the concentrations of lactic acid and tartaric acid were also higher, while the concentrations of maleic acid, acetic acid, and succinic acid were relatively low (Fig. 2). The total concentration of main organic acids secreted by the roots of 5-, 10-, 15-, and 20-year-old *L. thibetica* shrubs was 2 373.42 μg/mL, 1 659.23 μg/mL, 2 168.9 μg/mL, and 1 974.48 μg/mL, respectively.

Seasonal changes of organic acids secreted by shrub roots of different ages of *L. thibetica*: The concentration of organic acids secreted by the roots of *L. thibetica* shrubs in Tibet showed obvious seasonal changes, usually reaching the highest value in July and decreasing to the lowest in September (Fig. 3). In the same month of the growing season, the age of *L. thibetica* had a significant effect on the concentration of some organic acids ($P < 0.05$). The acetic acid concentration of 10-year-old *L. thibetica* shrub in August was significantly higher than that of other ages (Fig. 3*I*), while the maleic acid concentration of 5-year-old *L. thibetica* shrub in September was significantly lower than that of other ages (Fig. 3*H*). The concentration of malonic acid secreted by *L. thibetica* at different ages showed a downward trend with the passage of the growing season and decreased to the lowest in September (Fig. 3*E*), while the concentrations of maleic acid and succinic acid showed an upward trend at the end of the growing season (September) (Fig. 3*H*,*M*).

Relationship between acid secretion of shrub roots and soil water and heat in *L. thibetica*: The concentration of organic acids secreted by the roots of *L. thibetica* in Tibet is usually linearly correlated with soil moisture (Fig. 4). Among them, the concentrations of oxalic acid, tartaric acid, malic acid, acetic acid, citric acid and succinic acid are positively correlated with soil moisture (Fig. 4*A*,*C*,*E*,*H*,*K*,*M*), while the concentration of maleic acid is negatively correlated with soil moisture (Fig. 4*G*). In addition, the changes of lactic acid, malonic acid, and fumaric acid concentrations are less affected by soil moisture (Fig. 4*B*,*D*,*F*). Regression analysis with soil temperature showed that only the concentrations of malic acid, citric acid, and succinic acid showed a positive linear correlation with soil temperature (Fig. 5*E*,*K*,*M*), and the regression relationship between other organic acids and soil temperature was not significant (Fig. 5, $P > 0.05$).

Discussion

Composition of organic acids secreted by roots of *L. thibetica* shrub at different ages: As a key component of plant root exudates, organic acids play an important role in the interaction between plants and soil environment. The composition and content of organic acids are not only closely related to plant species (Gao and Jia, 2020), but also reflect the adaptation strategies of plants to specific environments. This study found that the organic acids secreted by the roots of *L. thibetica* shrubs in Tibet were mainly oxalic acid, lactic acid, and tartaric acid (Fig. 2), which was different from the results of tea trees and green poplars. Among them, the main organic acids in the root exudates of tea tree were citric acid, oxalic acid and acetic acid (Zahraeni and Uygur, 2024), while the organic acids with higher concentration in the root exudates of evergreen poplar were oxalic acid, succinic acid and citric acid (Wang et al., 2022). The differences in the composition of main organic acids secreted by different plant roots are affected by many factors. On the one hand, there are significant differences in metabolic pathways and efficiency among different plants, which directly determine their ability to synthesize organic acids. For example, plants in the vigorous growth stage tend to secrete more organic acids to meet the needs of rapid growth. On the other hand, different plant root morphological characteristics (such as surface area and tip number) have important effects on organic acid secretion potential. Studies have shown that the larger the root surface area and the more the number of tips, the stronger the ability of plants to secrete organic acids (Meier et al., 2020; Jiang

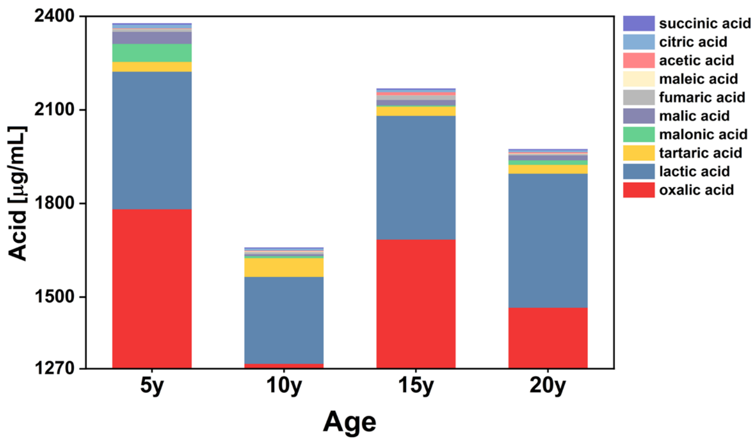

Fig. 2. Total concentration of organic acids secreted by roots of *Lonicera thibetica* at different ages in growing season. 5y, 10y, 15y, 20y represent 5 years, 10 years, 15 years, 20 years of *L. thibetica* shrubs.

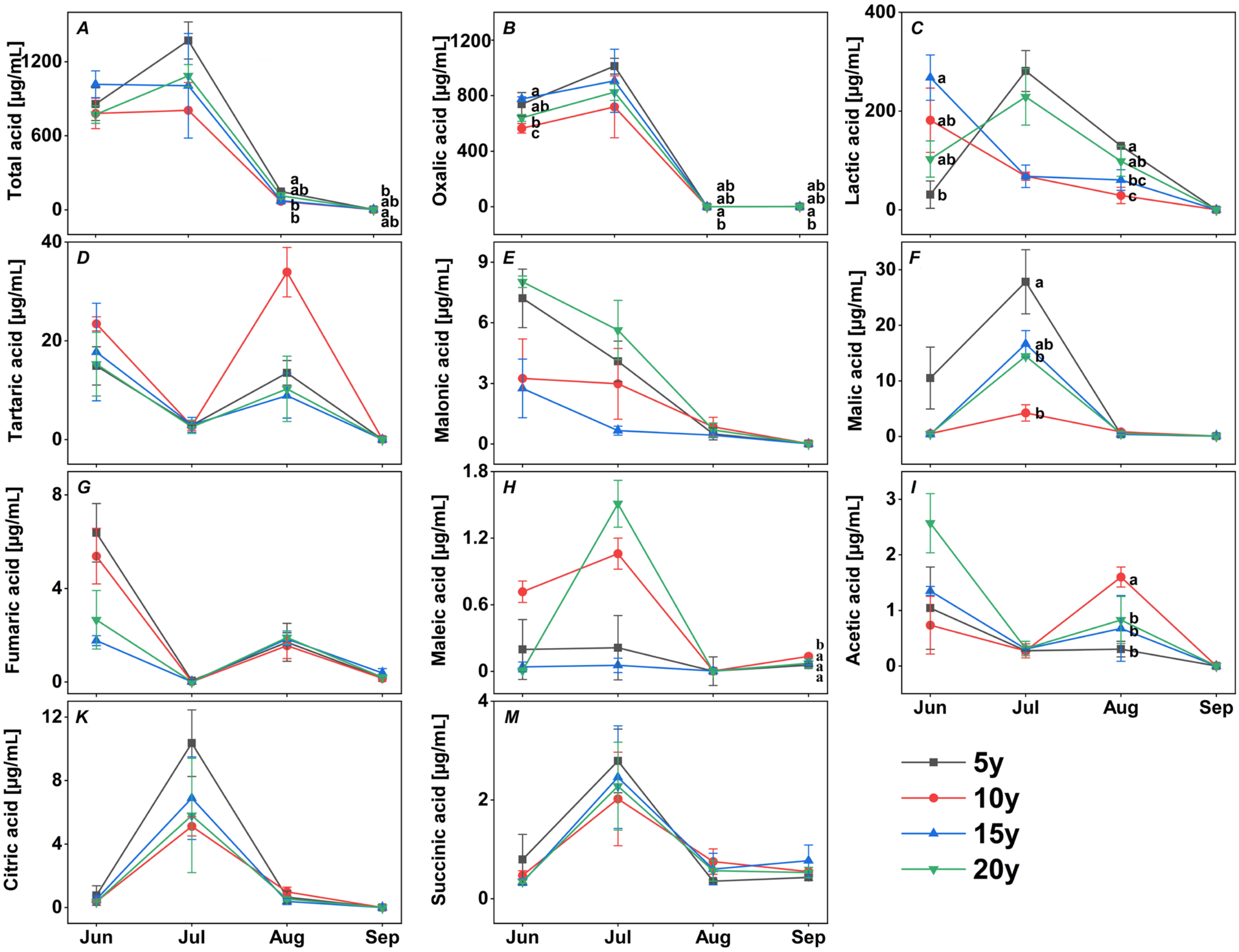

Fig. 3. Seasonal variation of organic acid concentration in root exudates of *Lonicera thibetica*. Means ± SEs (n = 4). Points bearing *lowercase letters* (P < 0.05) differ significantly between different ages as determined by LSD test, and the *unmarked points* represent no difference; 5y, 10y, 15y, 20y represent 5 years, 10 years, 15 years, 20 years of *L. thibetica* shrubs.

et al., 2022). In addition, the different types of mycorrhizal microorganisms also cause differences in the secretion of organic acids. This is because soil microorganisms are also involved in the regulation of the synthesis and release of organic acids through symbiosis with plants. This difference in secretion reflects the diversity of plant resource acquisition strategies (Wu et al., 2014). It is worth noting that the concentration of oxalic acid secreted by these tree roots is high, which may be due to the fact that oxalic acid can activate insoluble minerals in soil by chelating metal ions, acidifying soil and reducing high-valent iron, thus enhancing the absorption of nutrients by plants. This characteristic may reflect the 'convergent adaptation' mechanism formed in the long-term evolution of plants, that is, to optimize resource acquisition and enhance survival competitiveness by secreting specific organic acids (Zhalnina et al., 2018). The secretion patterns of different organic acids in plants reflect the adaptation strategies of plants in different habitats (Pan et al., 2013; Xu et al., 2013). Notably, oxalic acid (accounting for > 50%) mobilizes soil nutrients by dissolving insoluble minerals, while lactic acid supports microbial nutrient cycling under hypoxia – these exudate-soil interactions collectively facilitate long-term *L. thibetica* expansion (Van Hees and Lundström, 2000; Shahzad et al., 2015; Cai et al., 2024). The dominance of oxalic acid is primarily driven by its synthesis *via* the root glyoxylate cycle, a pathway requiring only two carbon skeletons per oxalic acid molecule, which makes it highly carbon-efficient and well-adapted to the low-nutrient stress of the Qinghai-Tibet Plateau (Gómez-Romero et al., 2024). Ecologically, this high oxalic acid secretion specifically enriches rhizospheric *Acidovorax* and *Pseudomonas*, further increasing available phosphorus (P) by 15 - 20% compared to surrounding grass patches (building on its mineral-dissolving capacity) and mildly acidifying the rhizosphere to inhibit the growth of competing grasses like *Kobresia setchwanensis* (Adeleke et al., 2017).

There was a significant difference in the concentration of organic acids released by the roots of *L. tibetica* shrubs at different ages at the same time (Fig. 3), which was consistent with the study on the root exudates of *Picea asperata* in the Miyaluo area of western Sichuan (Li et al.,

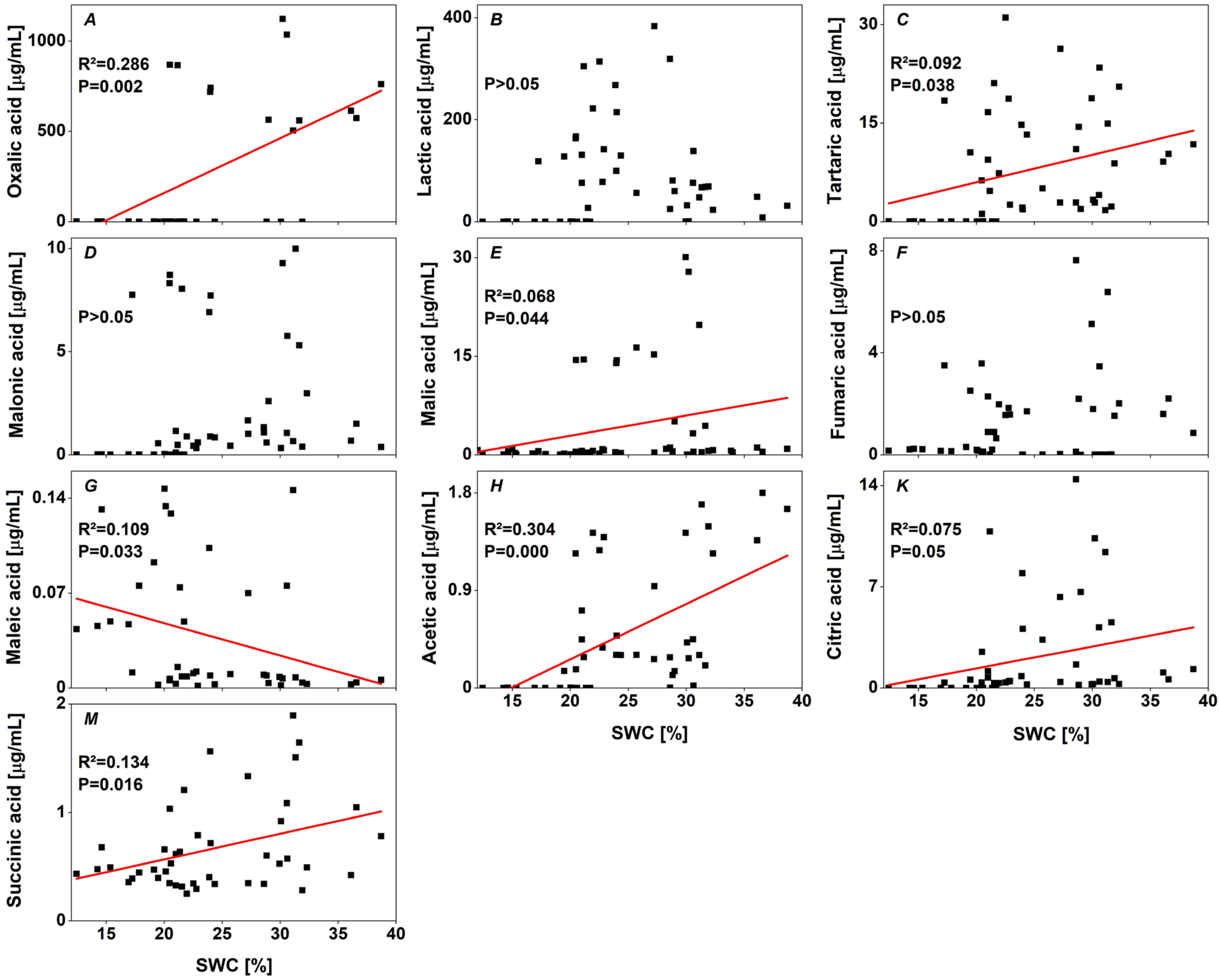

Fig. 4. Regression analysis of organic acid concentration and soil moisture content (SWC).

2014). This difference may be due to two reasons: on the one hand, this may be related to the growth status and metabolic capacity of trees of different ages. Young trees grow vigorously and metabolize actively, and tend to secrete more organic acids into the soil to promote soil nutrient absorption and resistance to biotic stress (Mao and Zhu, 2021). In contrast, older trees have slower metabolic rates and significantly reduce carbohydrate supply, resulting in lower organic acid secretion (Wu et al., 2020). On the other hand, the reasons for this difference are also closely related to the morphology and ecological functions of plants. With the increase of shrub age, the canopy expansion leads to the increase of precipitation interception and the decrease of soil water content. At the same time, the increase of plant biomass and transpiration further aggravated the decrease of soil moisture, thus inhibiting the secretion of organic acids in roots. This phenomenon indicates that there is a close feedback relationship between the morphological and physiological characteristics of plants and their living environment. Specifically, with age, plants dynamically adjust the secretion pattern of organic acids to balance resource acquisition and water-use efficiency (Acosta-Martínez et al., 2007). This adjustment not only affects plant survival strategies, but may also reshape the ecosystem nutrient cycle.

Seasonal dynamics of organic acids secreted by shrub roots of *L. thibetica* in Tibet: The concentration of organic acids secreted by the roots of *L. thibetica* in Tibet usually reaches a peak during the vigorous growth period (July) (Fig. 3). On the one hand, the seasonal variation of photosynthesis is a key factor affecting the secretion of organic acids. During the vigorous growth period, plants fix a large amount of carbon through photosynthesis (Giesler et al., 2007), and convert it into a large amount of organic acids through enzymatic reactions in the roots and release them to the rhizosphere (Jones, 1998). At this stage, the growth of fine roots was vigorous, and the metabolism of root epidermal cells was active, which further promoted the secretion of organic acids (Li et al., 2009). With the end of the growing season (September), although the overall metabolic activity of *L. thibetica* shrubs slowed down, the concentrations of maleic acid and succinic acid showed an upward trend (Fig. 3). The possible reason is that

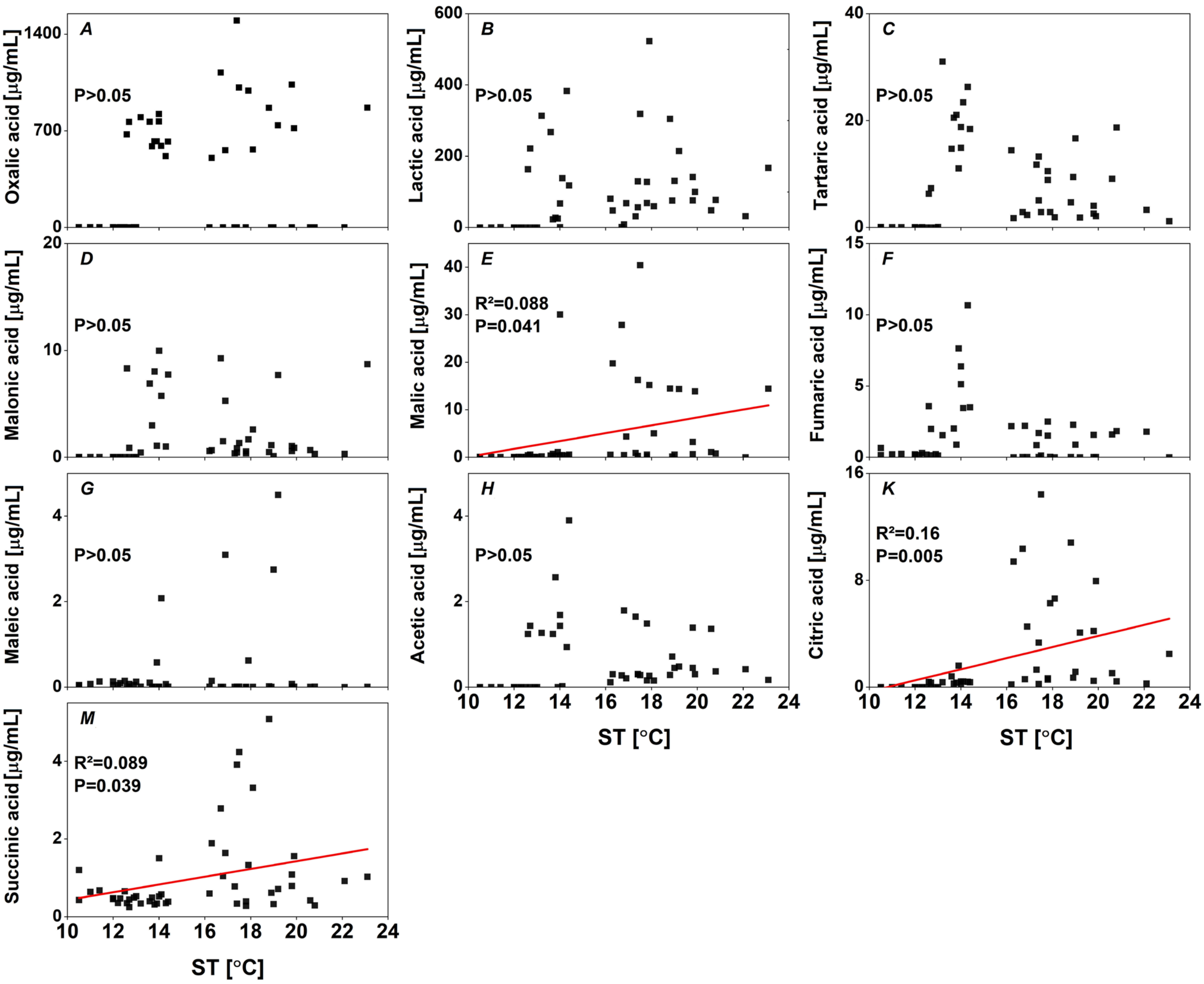

Fig. 5. Regression analysis of organic acid concentration and soil temperature (ST).

the soil temperature and moisture in this period inhibit the activity of enzymes, so that the ability of microorganisms (including mycorrhiza) attached to the roots to degrade organic acids is weakened (Fujii et al., 2012), resulting in the accumulation of organic acids in the rhizosphere, thereby increasing its concentration. These results suggest that plants adapt to seasonal environmental changes by actively regulating the secretion of organic acids, reflecting their adaptive mechanisms in carbon resource allocation and stress resistance improvement.

Relationship between acid secretion of shrub roots and soil water and heat in *L. thibetica*: Most of the organic acids secreted by the roots of *L. thibetica* were positively correlated with soil moisture content (Fig. 4), which was consistent with the results of many researchers (Dijkstra and Cheng, 2007; Zhao et al., 2016; Zhang et al., 2022). This phenomenon may be related to the physiological mechanism of plant root secretion. The root secretion is mainly concentrated in the 1 ~ 5 mm of the root tip region, and the root tip is also the main part of the plant to absorb water. Appropriate soil moisture is conducive to improving the activity of soil enzymes (such as phosphatase, urease, *etc.*). Soil enzymes enhance the metabolic activities of plants through signal transduction pathways, thereby promoting the synthesis and secretion of organic acids (Xie et al., 2009). However, it is interesting that the total amount of organic acids secreted by roots is the lowest in the shrub patches with the highest soil moisture content (Fig. 1, Fig. 2). The reason for this phenomenon may be that the soil moisture content of these shrub patches exceeds the maximum water threshold that can be absorbed by the root tip of *L. thibetica* shrub, resulting in hypoxia in the root tip. The hypoxic environment will inhibit the release of organic acids by root tip cells, thereby reducing the secretion of organic acids. As a key driving factor regulating the function of plant-soil system, soil moisture can promote the synergy between plant and soil microorganisms under appropriate conditions, promote nutrient cycling and ecosystem stability, but extreme water conditions may break this balance, resulting in decreased plant adaptability and impaired ecosystem function (Yu et al., 2024). In addition, there is a negative correlation between maleic acid concentration and soil moisture, which may be caused by an adaptation of plants to the environment. Plants respond to water stress by reducing the release of some organic acids, thereby reducing the metabolic burden and enhancing stress resistance and improving their viability (Zhang et al., 2023).

This study found that there was no significant correlation between most organic acid concentrations and temperature (Fig. 5). This is inconsistent with the results of the study in subalpine grasslands in Germany, which believed that under warming conditions, the demand for nitrogen and other nutrients in plants increased, thereby promoting the release of root exudates (Pausch and Kuzyakov, 2018). The reason for this difference may be related to the interference of other environmental factors such as soil moisture and soil type (Xiong et al., 2020; Wang et al., 2021). For example, excessive soil moisture may weaken the promoting effect of temperature increase on root exudates, and plants preferentially respond to water stress with limited resources rather than secretory activities (Dijkstra and Cheng, 2007). In addition, different soil types may also affect the physical and chemical properties of the rhizosphere environment, thereby changing the response pattern of plants to temperature changes (Acosta-Martínez et al., 2007). Besides soil moisture and temperature, soil pH and biotic interactions may also modulate acid secretion. Alpine meadow soils here are weakly acidic (pH 5.5 - 6.5; Xie and Gao, 2015), and pH deviations could alter organic acid chelating demand for metal ions (*e.g.*, Fe^{3+}), thus changing secretion patterns (Jiang et al., 2022). Additionally, ectomycorrhizal symbiosis may reduce plant reliance on acid secretion by enhancing nutrient absorption (Shahzad et al., 2015), though mycorrhizal colonization was not quantified here. In this study, the organic acids secreted by the roots of *L. thibetica* in Tibet were more significantly affected by soil moisture content, and the relationship with soil temperature was more complicated. This finding not only emphasizes the central role of soil moisture in regulating plant root secretion, but also reveals the high plasticity of plants in the face of changing environments. Notably, soil pH (modulable by dominant oxalic acid to alter nutrient solubility) and temperature-moisture synergy (Giesler et al., 2007) further refine this regulatory system, while lactic acid's support for hypoxic microbial cycling (Cai et al., 2024) links to moisture-induced hypoxia, adding unaddressed ecological layers.

Conclusion

Growing *L. thibetica* shrubs of varying ages in alpine meadows was the subject of this investigation into the seasonal changes in the composition of organic acids released by the roots and how these changes interacted with variations in soil temperature and moisture. Oxalic acid, lactic acid, and tartaric acid were the primary organic acids released by the roots of *L. thibetica*. Concentrations often followed a parabolic fluctuation pattern throughout the year, reaching a maximum during robust development and declining as shrubs aged. The ability of alpine shrubs to regulate the distribution of root organic acids is a survival mechanism that allows them to adjust to variations in the seasons and with age. There was a strong positive correlation between the concentration of organic acids secreted by alpine shrub roots and soil moisture. This suggests that precipitation is a significant climatic factor influencing the expansion of alpine shrubs, as soil moisture was a key component in the secretion process. To better understand how alpine shrub roots secrete organic acids and how these acids interact with soil chemical and microbiological properties, as well as how these acids change over time and what roles they play in ecosystems, further research is needed.

References

Acosta-Martínez, V., Cruz, L., Sotomayor-Ramírez, D. & Pérez-Alegría, L. (2007) Enzyme activities as affected by soil properties and land use in a tropical watershed. *Applied Soil Ecology*, 35, 35-45.

Adeleke, R., Nwangburuka, C. & Oboirien, B. (2017) Origins, roles and fate of organic acids in soils: A review. *South African Journal of Botany*, 108, 393-406.

Cai, Y., Yu, X., Zou, Y., Ding, S. & Min, Y. (2024) Shrubification of herbaceous peatlands modulates root exudates, increasing rhizosphere soil CO_2 emissions while decreasing CH_4 emissions. *Catena*, 245, 108282.

Cao, Q., Hu, J., Chen, X. et al. (2022) [Effects of vegetation restoration on soil carbon, nitrogen, phosphorus and ecological stoichiometry in sandy land of Northwestern Sichuan.] *Acta Agrestia Sinica*, 30, 523-531. [In Chinese]

Chen, M., Yao, X., Cheng, H. et al. (2023) Changes in Chinese fir plantations root exudation strategies seasonally and as tree age. *Forest Ecology and Management*, 545, 121239.

Dijkstra, F.A. & Cheng, W. (2007) Moisture modulates rhizosphere effects on C decomposition in two different soil types. *Soil Biology and Biochemistry*, 39, 2264-2274.

Fujii, Y., Dohmae, N., Takio, K. et al. (2012) A lectin from the mussel *Mytilus galloprovincialis* has a highly novel primary structure and induces glycan-mediated cytotoxicity of globotriaosylceramide-expressing lymphoma cells. *Journal of Biological Chemistry*, 287, 44772-44783.

Gao, X. & Jia, Y. (2020) [Analysis of organic acids in root exudates of desert steppe plants and their ecological effects.] *Journal of Ecology and Environment*, 29, 1927-1934. [In Chinese]

Gao, Y., Wang, H., Yang, F. et al. (2024) Relationships between root exudation and root morphological and architectural traits vary with growing season. *Tree Physiology*, 44, tpad118.

Gargallo-Garriga, A., Preece, C., Sardans, J., Oravec, M., Urban, O. & Peñuelas, J. (2018) Root exudate metabolomes change under drought and show limited capacity for recovery. *Scientific Reports*, 8, 12696.

Giesler, R., Högberg, M.N., Strobel, B.W., Richter, A., Nordgren, A. & Högberg, P. (2007) Production of dissolved organic carbon and low-molecular weight organic acids in soil solution driven by recent tree photosynthate. *Biogeochemistry*, 84, 1-12.

Gómez-Romero, M., Lindig-Cisneros, R., Cruzado-Vargas, A.N. & del-Val, E. (2024) Effects of organic acid application and ectomycorrhizal symbiosis on growth and morphological parameters of *Pinus pseudostrobus*. *Botanical Sciences*, 102, 843-853.

He, W., Yang, X., Xiao, J. et al. (2017) [Effects of nitrogen enrichment on root exudative carbon inputs in *Sibiraea*

angustata shrubbery at the eastern fringe of Qinghai-Xizang Plateau.] *Chinese Journal of Plant Ecology*, 41, 610-621. [In Chinese]
Jakoby, G., Rog, I., Megidish, S. & Klein, T. (2020) Enhanced root exudation of mature broadleaf and conifer trees in a Mediterranean forest during the dry season. *Tree Physiology*, 40, 1595-1605.
Jiang, Z., Thakur, M.P., Liu, R. et al. (2022) Soil P availability and mycorrhizal type determine root exudation in sub-tropical forests. *Soil Biology and Biochemistry*, 171, 108722.
Jones, D.L. (1998) Organic acids in the rhizosphere – a critical review. *Plant and Soil*, 205, 25-44.
Li, J., Jiang, X., Yin, H., Yin, C., Wei, Y. & Liu, Q. (2014) [Root exudates and soil microbes in three *Picea asperata* plantations with different stand ages.] *Journal of Applied Ecology*, 25, 325-332. [In Chinese]
Li, X., Yang, Z. & Li, Y. (2009) [Composition and dynamic changes of organic acids in root exudates of blueberry.] *Journal of Jilin Agricultural University*, 31, 578-580. [In Chinese]
Lian, J., Li, G., Zhang, J. & Massart, S. (2024) Nitrogen fertilization affected microbial carbon use efficiency and microbial resource limitations via root exudates. *Science of the Total Environment*, 950, 174933.
Macias-Benitez, S., Garcia-Martinez, A.M., Caballero Jimenez, P., Gonzalez, J.M., Tejada Moral, T. & Parrado Rubio, J. (2020) Rhizospheric organic acids as biostimulants: monitoring feedbacks on soil microorganisms and biochemical properties. *Frontiers in Plant Science*, 11, 633.
Mao, M. & Zhu, F. (2021) [Research progress and prospect of root exudate-mediated plant stress resistance.] *Chinese Journal of Ecological Agriculture*, 29, 1649-1657. [In Chinese]
Meier, I.C., Avis, P.G. & Phillips, R.P. (2013) Fungal communities influence root exudation rates in pine seedlings. *FEMS Microbiology Ecology*, 83, 585-595.
Meier, I.C., Tückmantel, T., Heitkötter, J. et al. (2020) Root exudation of mature beech forests across a nutrient availability gradient: the role of root morphology and fungal activity. *New Phytologist*, 226, 583-594.
Pan, K., Xu, L., Wu, F., Han, Z., Chu, L.R. & Qiu, C.F. (2013) Fungicidal effects of wheat root exudates on *Fusarium oxysporum* f. sp. *niveum*. *Allelopathy Journal*, 32, 257-266.
Pausch, J. & Kuzyakov, Y. (2018) Carbon input by roots into the soil: Quantification of rhizodeposition from root to ecosystem scale. *Global Change Biology*, 24, 1-12.
Shahzad, T., Chenu, C., Genet, P. et al. (2015) Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species. *Soil Biology and Biochemistry*, 80, 146-155.
Van Hees, P.A.W. & Lundström, U.S. (2000) Equilibrium models of aluminium and iron complexation with different organic acids in soil solution. *Geoderma*, 94, 201-221.
Wang, C., Gao, Q. & Yu, M. (2019) Quantifying trends of land change in Qinghai-Tibet Plateau during 2001-2015. *Remote Sensing*, 11, 2435.
Wang, Q., Chen, L., Xu, H. et al. (2021) The effects of warming on root exudation and associated soil N transformation depend on soil nutrient availability. *Rhizosphere*, 17, 100263.
Wang, Y., Chen, X., Lou, S. et al. (2018) [Invasion of grassland scrub: process, mechanism and effect.] *Acta Prataculturae Sinica*, 27, 219-227. [In Chinese]
Wang, Y., Feng, F., Ge, J., Li, Y. & Yu, X. (2022) [Effects and mechanisms of plant root exudates on soil remediation.] *Acta Ecologica Sinica*, 42, 829-842. [In Chinese]
Wasyliw, J. & Karst, J. (2020) Shifts in ectomycorrhizal exploration types parallel leaf and fine root area with forest age. *Journal of Ecology*, 108, 2270-2282.
Weinhold, A., Döll, S., Liu, M. et al. (2022) Tree species richness differentially affects the chemical composition of leaves, roots and root exudates in four subtropical tree species. *Journal of Ecology*, 110, 97-116.
Wu, H., Xiang, W., Ouyang, S. et al. (2020) Tree growth rate and soil nutrient status determine the shift in nutrient-use strategy of Chinese fir plantations along a chronosequence. *Forest Ecology and Management*, 460, 117896.
Wu, L., Lin, X., Lin, W. (2014) [Advances and perspective in research on plant-soil-microbe interactions mediated by root exudates.] *Chinese Journal of Plant Ecology*, 38, 298-310. [In Chinese]
Xie, M., Yan, C., Ye, J. & Wei, L. (2009) Impact of phenanthrene on organic acids secretion and accumulation by perennial ryegrass, *Lolium perenne* L., root. *Bulletin of Environmental Contamination and Toxicology*, 83, 75-80.
Xie, Q. & Gao, Y. (2015) [Effects of freeze-thaw on soil carbon, nitrogen and phosphorus availability in alpine meadow of Qinghai-Tibet Plateau.] *Journal of Soil and Water Conservation*, 29, 137-142. [In Chinese]
Xiong, D., Huang, J., Yang, Z. et al. (2020) The effects of warming and nitrogen addition on fine root exudation rates in a young Chinese-fir stand. *Forest Ecology and Management*, 458, 117793.
Xu, W., Wu, F., Chang, C., Liu, S. & Zhou, Y. (2013) Effects of wheat as companion cropping on growth, soil enzymes and disease resistance of watermelon. *Allelopathy Journal*, 32, 267-278.
Yang, Z., Meng, L., Liu, Z. et al. (2024) Warming enhances the negative effects of shrub removal on phosphorus mineralization potential. *Science of the Total Environment*, 924, 171517.
Yu, C., Mawodza, T., Atkinson, B.S. et al. (2024) The effects of soil compaction on wheat seedling root growth are specific to soil texture and soil moisture status. *Rhizosphere*, 29, 100838.
Zahraeni, N.A. & Uygur, V. (2024) Effect of low molecular weight organic acids and liming on phosphorus adsorption in acidic tea soils in the Black Sea Region, Türkiye. *Communications in Soil Science and Plant Analysis*, 55, 3161-3178.
Zhalnina, K., Louie, K.B., Hao, Z. et al. (2018) Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology*, 3, 470-480.
Zhang, X., Yan, J., Rahman, M.K.U. & Wu, F. (2022) The impact of root exudates, volatile organic compounds, and common mycorrhizal networks on root system architecture in root-root interactions. *Journal of Plant Interactions*, 17, 685-694.
Zhang, X., Bilyera, N., Fan, L. et al. (2023) The spatial distribution of rhizosphere microbial activities under drought: water availability is more important than root-hair-controlled exudation. *New Phytologist*, 237, 780-792.
Zhao, D., Zhu, Y., Wu, S. & Zheng, D. (2021) Projection of vegetation distribution to 1.5°C and 2°C of global warming on the Tibetan Plateau. *Global and Planetary Change*, 202, 103525.
Zhao, K., Zhou, B., Ma, W. & Yang, L. (2016) [Research progress on the effects of different environmental stresses on the secretion of organic acids by roots.] *Soil*, 48, 235-240. [In Chinese]