Does the wetland invader, Alternanthera philoxeroides cause different ecological impact compared to the native Typha angustifolia? A comparison of sediment stoichiometry and microbial pattern between urban and periurban rivers

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Research Article

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Abstract

Background and Aims Plant invasion can modify habitat characteristics for instance soil stoichiometry and microbial pattern. However, few studies concerned the effects of plant invasion on the soil properties in the urban ecosystem. The present study aims to explore the impact of aquatic plant invasion on sediment properties within the urbanization context.

Methods First, population density and impervious surface area, were used to construct an urbanization gradient of river wetlands and divide the two rivers of Qingdao City, China – Zhangcun River and Wenquan River into urban and periurban types. Second, sediment samples were collected from the plots invaded by the aquatic plant invader, *Alternanthera philoxeroides* and its native neighbor, *Typha angustifolia* in urban and periurban rivers. Lastly, sediment properties were determined and a comparison was performed.

Results First, a general similar fertility and stoichiometry was found between the sediment derived from the invasive and that from the native. Second, a higher bacterial diversity was found in the sediment derived from *A. philoxeroides* merely in the periurban river, while the higher bacterial diversity was merely shown for the Shannon's diversity index of *A. philoxeroides* in the urban river. Third, *A. philoxeroides* shaped a novel soil microbial structure since more microbes relevant with nutrient cycling were accumulated compared to the native. Lastly, urbanization gradient affected the comparison between the invasive and native plants on soil properties.

Conclusion

The invasion of exotic aquatic plant altered the sediment microbial pattern to some extent and the potential plant-soil feedback needs further investigation.

Introduction

As a crucial component of global environmental change, biological invasion of alien plants has induced diverse impacts on ecosystems (Parra-Tabla and Arceo-Gómez 2021; Tasker et al. 2022). Once researches on the impact of exotic invasive plants have primarily focused on aboveground ecology such as the alteration of native plant community composition and diversity; however, recent studies show increasing interests to the "invisible" belowground (Zhang et al. 2020; Unger et al. 2022). Considering belowground impacts, exotic invasive plants have been reported to modify the chemical and biological properties of soil through processes such as physical intrusion and release of secondary metabolites (Zhou and Staver 2019; Kalisz et al. 2021; Wen et al. 2021). Exotic invasive plants have been demonstrated to affect soil properties including carbon pool, nutrient availability and microbial activity (Duda et al. 2003; Chapuis-Lardy et al. 2006; Bills et al. 2010; Zubek et al. 2016; Stefanowicz et al. 2016; Majewska et al. 2018; Zhou and Staver 2019; Li et al. 2022). Field observations and experiments have both shown that the invasion of exotic plants can alter the soil stoichiometry and promote larger nutrient pools compared to native plants (Elgersma 2014). For instance, nutrient levels of soil nitrogen and

phosphorus increased along with the increasing cover of *Halogeton glomeratus*, which invaded a native winterfat community (Duda et al. 2003). Plots invaded by *Solidago gigantea* had higher labile phosphorus concentrations compared to the adjacent sites occupied by the native grassland vegetation (Chapuis-Lardy et al. 2006). The region invaded by reed canary grass (*Phalaris arundinacea*) had a larger soil organic carbon pool compared to areas colonized either by a native sedge *Scirpus cyperinus* or a mixed assemblage of 22 native species in a south-central Indiana (USA) wetland (Bills et al. 2010). Considering soil microbial biota, the invasion of alien plants has been proved to enhance microbial diversity, downgrade the soil microbial quality or reduce the soil microbial activity of arbuscular mycorrhizal fungi and the abundance of sulfur-oxidizing functional genes (Duda et al. 2003; Zubek et al. 2016; Stefanowicz et al. 2016; Majewska et al. 2018; Zhou and Staver 2019; He et al. 2021). Exotic invasive plants may modify the soil pathogen communities and the underlying plant-soil feedbacks potentially influences the invasion process (Elgersma 2014). Eppinga et al. (2006) demonstrated that the growth of exotic species which gather more pathogens or herbivores is promoted if those exotic species can tolerate pathogens or herbivores, relative to the native plants. Mangla et al. (2008) found that the tropical invasive weed, *Chromolaena odorata* accumulated great abundance of the generalist soil borne fungi, *Fusarium*, which induced a negative feedback to native plant species. Accumulation of local pathogen hypothesis, as a refinement of the above findings, suggests that the successfully established alien plants could congregate native pathogens and thus impede the growth and dispersal of native plants (Ravichandran and Thangavelu 2017).

Urban ecosystem is highly correlated with the development of human civilization since the majority of global population have already lived in urban regions – expected to reach 60% by 2030 (United Nations 2013). Urban ecosystem suffers severe alien plant invasion since human activities such as ornamental horticulture and phytoremediation promote the introduction of alien plants; meanwhile, artificial disturbances such as eutrophication and traffic construction facilitate their establishment and dispersal (Kalwij et al. 2008; Cadotte et al. 2017; Hulme et al. 2019; van Kleunen et al. 2018). Due to the discrepancy in the developmental status within the urban area, an urban-periurban gradient usually evolves under the urbanization gradient framework (McKinney 2002; McDonnell et al. 2008; Calfapietra et al. 2015; Scherr and Jamieson 2021). The urban-periurban gradient can be fundamentally destructured through two proxies – population density and impervious surface area – the area covered by roads, buildings and other man-made infrastructures (McKinney 2002; McDonnell et al. 2008). Commonly, a higher population density and a higher percentage of impervious surface area are possessed by urban area than by periurban region, which usually causes the alleviation of anthropogenic disturbance from urban to periurban area (McKinney 2002; McDonnell et al. 2008). As a cascading effect, the pollutant discharge is likely more intense in urban area than in periurban region, which may cause more eutrophic soil and water in the urban area (McKinney 2002). The urban-periurban gradient has been frequently explored in urban ecology, however, the application of this gradient in invasion ecology has been limited (McDonnell et al. 2008; Kowarik 2008). Most former researches, which integrated the use of urban-periurban gradient into the exploration of invasion ecology, have shown that the urban-periurban gradient affects the diversity and distribution pattern of alien plants and usually a more congregated distribution

and a higher diversity of alien plants existed in the urban region rather than in the periurban area (Liendo et al. 2016; Cadotte et al. 2017; Grella et al. 2018). Current findings on the invasion ecology of urban alien plants generally corresponds to the disturbance hypothesis of invasion mechanism as disturbance causes the resource fluctuation, modify the energy flux and reconfigure the niches and thus promote the invasion of alien plants (Planillo et al. 2021). Due to a more intensified anthropogenic disturbance in the urban region, a severer invasion of alien plants has existed in the urban region rather than periurban area (Cadotte et al. 2017; Grella et al. 2018). Therefore, the impact of alien plant invasion may be different between urban and periurban regions. However, the impacts of invasive alien plants on the urban ecosystem along the urban-periurban gradient have been little studied.

Urban wetland including urban rivers, streams, ponds and lakes is a key component of urban ecosystem since most ancient civilization and modern cities were born from wetlands (Dong 2018). Urban wetland is a major recipient of alien macrophytes since the release of propagules from aquariums and the intentional introduction of alien ornamental plants for horticulture accelerate the expansion of alien macrophytes (Cadotte et al. 2017; van Kleunen et al. 2018; Hulme et al. 2019). However, the ecology of macrophytes in the urban region has been little probed (Paul and Meyer 2008). In recent years, the Chinese government has aimed at building a harmonious ecological civilization both in urban and rural area. In order to execute this strategy, the introduction of exotic ornamental aquatic plants for landscape architecture and the re-vegetation of green and blue spaces has been vigorous across the country. However, the introduction accelerates the invasion of alien plants in urban area, which may cause a series of ecological problems such as the degradation of native biodiversity and the obstruction of city channels (Moro and Castro 2015; Oertli et al. 2018). Hence, the impact of alien macrophytes on urban wetland is worthy of study. *Alternanthera philoxeroides* has a long invasion history in China and previously this species has been expansive mainly in suburbs and rural area of subtropical region. However, this species has been pervasive in the urban wetlands of warm temperate region in China. Ramsar Convention has proposed the definitions of urban and periurban wetlands based on the urban-periurban gradient (Ramsar 2008). However, little is known about how the impact of invasive alien macrophytes on wetland soil including carbon pool, fertility, stoichiometry and microbial diversity, assemblage, structure and function along the urban-periurban gradient (Tasker et al 2022). In the present study, the impact of the nuisance invasive macrophyte, *A. philoxeroides* on wetland soil was studied in two river wetlands with different urbanization contexts. To evaluate the invasion impact, the soil chemical and biological properties were compared between invasive-derived and native-derived soils. The following hypotheses were postulated:

1. The invasive *A. philoxeroides* enhances soil fertility and microbial diversity, and converge more beneficial soil microbes compared to the native *T. angustifolia*;
2. The above effects are different between urban and periurban river wetlands.

Materials And Methods

Sampling area

Two river wetlands – Zhangcun River and Wenquan River of Qingdao City, Shandong Province, China were selected as the sampling area for the present study (the climatic factors see Table 1; Fig. 1; Fig. 2). Zhangcun River is located in the core residential region of Laoshao District, while Wenquan River is located in the sparsely populated suburb of Jimo District. The data of population density of Qingdao city at 10 a.m. on weekdays was obtained from Gaode Map (AutoNavi Software Co., Ltd., https://www.amap.com/) by API (Application Programming Interface). Gaode is one of the most famous navigation and location-based services provider in China. The population heatmap was then generated through Kernel Density method using ArcGIS 10.3 software(Environmental Systems Research Institute, California). The population heatmap shows that the population density was higher around the sampling section of Zhangcun River than that of Wenquan River (Fig. 1). Google imagery with 1 m spatial resolution of the two river sampling areas was downloaded from the Google Earth service using the BIGEMAP software (http://www.bigemap.com). 1 km buffer zone was created for each area based on the river bank and the land-use/cover was classified into four types of road, river, construction, and green space based on the visual interpretation method using ArcGIS 10.3 software (Environmental Systems Research Institute, California). The ratio of impervious surface area to pervious surface area is 6.22:1 for the 1 km range along the sampling section of Zhangcun River, while this ratio is 0.89:1 for the 1 km range along the sampling section of Wenquan River (Fig. 2). Based on the evaluation of population density and impervious surface area, the urbanization intensity is greater in the sampling section of Zhangcun River than that of Wenquan River (McKinney 2002; McDonnell et al. 2008). Therefore, Zhangcun River and Wenquan River are separately classified into urban and periurban river wetland types.

Table 1
Climatic factors of the two sampling sites

	Latitude (°N)	Longitude (°E)	MAP (mm)	MAT (°C)	HT (°C)	LT (°C)
Zhangcun River	36.1267	120.4597	73.9	12.5	24.0	0.2
Wenquan River	36.4404	120.6561	73.8	12.3	23.9	-0.5

MAP – mean annual precipitation, MAT – mean annual temperature, HT – highest temperature, LT – lowest temperature

Sampling Approach

A total of 31 quadrats of macrophyte populations were sampled in these two rivers – 8 quadrats of *Alternanthera philoxeroides and* 4 quadrats of *Typha angustifolia* were investigated in the sampling section of Zhangcun River, and 11 quadrats of *A. philoxeroides* and 8 quadrats of *T. angustifolia* were monitored in the sampling section of Wenquan River. *T. angustifolia* is the native species co-occuring with the exotic invasive *A. philoxeroides* in these two rivers. The quadrat size is 1 m × 1 m within the center of respective pure mats of *A. philoxeroides* and mono-stands of *T. angustifolia* (Müllerová et al.

2020). The distance between any two quadrats of the same species is at least 20 m in order to avoid sampling of the same genet (Oduor et al. 2022).

The sediment underneath each plant of each quadrat was carefully collected beneath the center of quadrat surface (approximately 20 cm-deep from the surface). The sediment was immediately placed into labelled plastic sampling bags and 2-ml centrifuge tubes. Then, the sampling bags and centrifuge tubes were put into an ice box. The sediment in the sampling bags were naturally withered and then the dry materials were used for the elemental analyses of carbon, nitrogen and phosphorus. The centrifuge tubes were placed into a box with dry ice and then sent to Novogene Co. Ltd for the 16S rDNA amplicon sequencing analysis of soil microbial diversity and function.

Soil Elemental Analysis

The soil carbon and nitrogen contents (TC and TN) were measured using a EuroEA3000 CHNS-O analyser (EuroVector, Italy). The soil phosphorus content (TP) was determined according to National Standards of the People's Republic of China: method for determination of soil total phosphorus (GB 9837–88). Then, soil C/N, soil C/P and soil N/P was calculated. A one-way ANOVA with Tukey’s test was performed to detect the probable difference in soil TC, soil TN, soil TP, soil C/N, soil C/P and soil N/P between different species in respective urban and periurban river using SPSS 22.0 (SPSS, Chicago, IL, USA).

Sequencing

Extraction of genome DNA

Total genome DNA from samples was extracted using CTAB method. DNA concentration and purity was monitored on 1% agarose gels. According to the concentration, DNA was diluted to 1ng/μL using sterile water.

Amplicon Generation

16S rRNA/18SrRNA/ITS genes of distinct regions (16S V4/16S V3/16S V3-V4/16S V4-V5, 18S V4/18S V9, ITS1/ITS2, Arc V4) were amplified used specific primer (e.g. 16S V4: 515F806R, 18S V4: 528F-706R, 18S V9: 1380F-1510R, et. al) with the barcode. All PCR reactions were carried out with 15 μL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs); 2 μM of forward and reverse primers, and about 10 ng template DNA. Thermal cycling consisted of initial denaturation at 98℃ for 1 min, followed by 30 cycles of denaturation at 98℃ for 10 s, annealing at 50℃ for 30 s, and elongation at 72℃ for 30 s. Finally 72℃ for 5 min.

Pcr Products Quantification And Qualification

Mix same volume of 1X loading buffer (contained SYB green) with PCR products and operate electrophoresis on 2% agarose gel for detection. PCR products was mixed in equidensity ratios. Then, mixture PCR products was purified with Qiagen Gel Extraction Kit (Qiagen, Germany).

Library preparation and sequencing Sequencing libraries were generated usingTruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. At last, the library was sequenced on an Illumina NovaSeq platform and 250 bp paired-end reads were generated.

Data analysis

Paired-end reads assembly and quality control

Data split

Paired-end reads was assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence.

Sequence Assembly

Paired-end reads were merged using FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/), a very fast and accurate analysis tool, which was designed to merge paired-end reads when at least some of the reads overlap the read generated from the opposite end of the same DNA fragment, and the splicing sequences were called raw tags.

Data Filtration

Quality filtering on the raw tags were performed under specific filtering conditions to obtain the high-quality clean tags according to the QIIME(V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html) quality controlled process.

Chimera Removal

Chimera removal
The tags were compared with the reference database (Silva database, using UCHIME algorithm (UCHIME Algorithm, http://www.drive5.com/usearch/manual/uchime_algo.html) to detect chimera sequences, and then the chimera sequences were removed. Then the Effective Tags finally obtained.

Otu Cluster And Species Annotation

OTU Production

Sequences analysis were performed by Uparse software (Uparse v7.0.1001, http://drive5.com/uparse/). Sequences with ≥ 97% similarity were assigned to the same OTUs. Representative sequence for each OTU was screened for further annotation.

Species Annotation

For each representative sequence, the Silva Database (http://www.arb-silva.de/) was used based on Mothur algorithm to annotate taxonomic information.

Data Normalization

OTUs abundance information were normalized using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity was performed based on this output normalized data.

Alpha Diversity

Alpha diversity is applied in analyzing complexity of species diversity for a sample through four indices including observed species, chao1, Shannon's diversity index and Simpson's diversity index. The four indices in our samples were calculated with QIIME (Version 1.7.0) and displayed with R Software (Version 2.15.3).

Results

Soil fertility and stoichiometry

Generally, no significant differences were shown for the soil TC, TN and TP and soil stoichiometry – soil C/N, C/P and N/P between the invasive plant-derived soil and native plant-derived soil regardless of river type (Fig. 3a, c, d, e and f; Fig. 4a, b, c, e and f). However, the soil TN derived from *A. philoxerides* was significantly lower than that of the soil derived from *T. angustifolia* in Zhangcun river (urban) (Fig. 3b), while the soil C/N was significantly lower underneath *A. philoxeroides* than underneath *T. angustifolia* in Wenquan River (periurban) (Fig. 4d).

Soil Bacteria

No significant differences between the soil derived *A. philoxeroides* and that derived from *T. angustifolia* existed in observed species, chao1, Shannon's diversity index and Simpson's diversity index in Zhangcun river (urban) (Fig. 5a, b, c and d). However, significantly higher values in observed species, chao1,

Shannon's diversity index were observed in the soil derived *A. philoxeroides* than that derived from *T. angustifolia* (Fig. 6a, b and c). Despite insignificant, the significance of difference in Simpson's diversity index between the soil derived from *A. philoxeroides* and that derived from *T. angustifolia* reached 0.075 (Fig. 6d).

The structure and relative abundance of top 10 bacterial genera was similar for the soil derived from *A. philxeroides* and that from *T. angustifolia* in Zhangcun River (urban) (Fig. 7). However, *A. philoxeroides* accumulated a higher relative abundance of *Denitratisoma* and *Candidatus methylomirabilis* than *T. angustifolia* in Wenquan River (periurban) (Fig. 8).

Soil Fungi

No significant differences between the soil derived *A. philoxeroides* and that derived from *T. angustifolia* existed in observed species, chao1 and Simpson's diversity index, while a significant higher values in Shannon's diversity index was shown for the soil derived from *A. philoxeroides* regardless of river type (Fig. 9a, b, c and d; Fig. 10a, b, c and d).

In Zhangcun River (urban), *A. philoxeroides* gathered a higher relative abundance of *Russula*, while *T. angustifolia* gathered a higher relative abundance of *Blumeria* (Fig. 11). In Wenquan River (periurban), *A. philoxeroides* accumulated a higher relative abundance of *Leucangium* and *Auriculariales*, while *T. angustifolia* accumulated a higher relative abundance of *Russula* (Fig. 12).

Discussion

The fertility and stoichiometry were generally similar between the soil derived from the invasive plant and that derived from the native neighbor irrespective of urbanization gradient

Several researchers found that a higher fertility (e.g. richer N and P availability) and a larger carbon pool were shown in the sites invaded by exotic plants (Majewska et al. 2018; Zhou and Staver 2019; Li et al. 2022). Further studies on the mechanism revealed that the exotic plants altered the nutrient level of invaded sites through physiological and physicochemical processes such as the secretion of root exudates and litter decomposition (Zhou and Staver 2019; Kalisz et al. 2021; Wen et al. 2021). However, prior studies focused on how the invasion of exotic plants alter the nutrient availability of terrestrial soil, which is different from that of wetland sediment. Since the wetland is immersed or saturated with water, the physicochemical properties of water determine the sediment fertility to a large extent. For instance, the flow velocity and water depth influence the nutrient deposition in the sediment (Johnston 1991). Moreover, the secretion of root exudates and litter decomposition may be influenced by the water physicochemical properties such as water trophic status and water flow (Bastias et al. 2020; Yue et al. 2022). For instance, the nutrient releases in the process of litter decay however the water flow likely affects the movement and deposition of the released nutrients in the sediments (Dalu et al. 2019). Hence,

water is likely an interfering factor in the fertility and stoichiometry of invasive and native plants, which may cause the consistency in the fertility and stoichiometry between invasive and native plants.

Intriguingly, the differences of urbanization level between the two sampled river wetlands did not induce the inconsistency in the comparison of fertility and stoichiometry between the soil derived from the invasive *A. philoxeroides* and its native neighbor, *T. angustifolia* in general. In the light of disturbance hypothesis, urbanization usually causes the intensification of anthropogenic disturbance and thus promote the invasion of exotic plants (Planillo et al. 2021). Beneath this assumption, the invasion of exotic plants in the urban region should have greater ecological impacts than that in the periurban area. However, using wetland aquatic plants as the model, the present study found that the impacts on the comparison of soil fertility and stoichiometry between invasive and native plants were mostly similar within the urbanization gradient framework. We speculate that water acts as a barrier to mitigate the anthropogenic disturbance from urban land. River flows may mitigate and dissipate the disturbance such as the exogenous nutrients from the land runoff (Johnston 1991; Dalu et al. 2019).

Despite the overall similarity, a tiny difference in fertility and stoichiometry between the sediments derived from invasive and native plants existed. In urban river, soil TN was significantly lower for the sediment derived from *A. philoxeroides* than that derived from *T. angustifolia.* In periurban river, soil C/N was significantly lower for the sediment derived from *A. philoxeroides.* Since sediment fertility (e.g. soil TN) and stoichiometry (e.g. soil C/N) determine the relative abundance of soil nutrients (e.g. N) and thus the resource availability of habitats, the effects of *A. philoxeroides* invasion on sediment nitrogen availability reversed to some extent. A positive plant-soil feedback tends to exist for *A. philoxeroides* in less disturbed periurban sites rather than more disturbed urban sites compared to *T. angustifolia*, since the relative nitrogen availability was higher for the soil derived from *A. philoxeroides* in the periurban river (Duda et al. 2003; Chapuis-Lardy et al. 2006; Elgersma 2014).

A partial divergence in soil bacterial diversity but a overall convergence in soil fungal diversity between the invasive A. philoxeroides **and the native** T. angustifolia

The present study found that the soil derived from the invasive *A. philoxeroides* possessed a higher soil bacterial α-diversity than that derived from its native neighbor, *T. angustifolia* in the periurban river. The first hypothesis – exotic plant invasion enhances soil microbial diversity, was partially demonstrated by the above result and has been also supported by several previous studies (Duda et al. 2003; Zhou and Staver 2019; He et al. 2021). However, a consistency in soil bacterial diversity was shown for the sediments derived from the two invasive and native plants in the urban river. Therefore, the comparison between invasive and native plants on soil bacterial α-diversity shifted along the urban-periurban gradient, which corresponds to the second hypothesis in part. Since urban region commonly possesses a higher population density and a larger impervious surface including roads and buildings than periurban area, anthropogenic disturbance usually weakens along the urban-periurban gradient (McKinney 2002; McDonnell et al. 2008). Thus, urban river is likely a larger sink of land runoff, sewage discharge and pollutant deposition than periurban river (McKinney 2002). These external nutrient input may neutralize

the effects of invasive plants on soil microbes through processes such as root exudation and litter decomposition. Correspondingly, the effects of root exudation and litter decomposition may be more prominent in the periurban river since the periurban river suffered a lower intensity of anthropogenic disturbance than the urban river. Disturbance has been certified to promote the growth of microbes, which likely neutralizes the discrepant impacts of invasive and native plants.

Intriguingly, a general similarity in fungal diversity existed between the soil derived from the invasive *A. philoxeroides* and that from the native *T. angustifolia* within both urban and periurban contexts. Despite that several studies found that exotic plant invasion altered the soil fungal diversity, it is noteworthy that the alteration could be increasing or decreasing and the targeted invasive plants in these studies were tall herbs (e.g. *Impatiens glandulifera* and *Spartina alterniflora*) or trees (e.g. *Pinus nigra*) with dense developed belowground parts (Gaggini et al. 2018; Zhang et al. 2021; Sapsford et al. 2022). *A. philoxeroides* has a weak root system relative to the above species and its effects on soil fungi are probably limited. Despite that, *A. philoxeroides* showed a general similar soil fungal α-diversity compared to the native tall herb *T. angustifolia* and even a higher value of Shannon's diversity index. Thus, the effects of *A. philoxeroides* invasion on fungal diversity might be relatively strong when considering the discrepancy in life forms for the two species. Moreover, the comparison on soil fungal diversity between invasive and native plants did not differ along the urban-periurban gradient, which partially opposes the second hypothesis. Since anthropogenic disturbance usually weakens along the urban-periurban gradient, the identical comparative results of soil fungal diversity may be resulted from a stable response of fungi to disturbance (Osburn et al. 2019; Chen et al. 2020)

Alternanthera philoxeroides **shaped a novel soil microbial structure compared to native neighbors**

Considering soil bacteria, the invasive *A. philoxeroides* gathered more abundant genera of *Denitratisoma* and *Candidatus methylomirabilis* in the soil compared to *T. angustifolia* in the periurban river, which corresponds to the first hypothesis to some extent. *Denitratisoma* and *Candidatus methylomirabilis* are involved in the biogeochemical cycle, and are demonstrated to be correlated with the denitrification and nitrate reduction (Luesken et al. 2012; Cruz et al. 2021). Soil nitrogen availability significantly affects the plant performance (Cruz et al. 2021). The abundance of *Denitratisoma* and *Candidatus methylomirabilis* may determine the soil nitrogen availability since a larger abundance of *Denitratisoma* and *Candidatus methylomirabilis* may induce a greater loss of soil nitrogen availability through the disoxidation of nitrate ions (e.g. NO^{3-} and NO^{2-}) to N_2 (Luesken et al. 2012; Fu et al. 2020; Cruz et al. 2021). The denitrification process likely downgrade the habitat hospitability for plant growth. Through this approach, *A. philoxeroides* may impede the intrusion of the native neighbor such as *T. angustifolia* into its populations and thus maintain the population stability. In a sense, the *Denitratisoma* and *Candidatus methylomirabilis* probably construct a safeguard for *A. philoxeroides* to resist the encroachment of other species.

However, the structure was similar for the two invasive and native plants in the urban river. The comparison between invasive and native plants on soil bacterial structure shifted along the urban-

periurban gradient, which corresponds to the second hypothesis. Urbanization seems to promote the convergence in soil bacterial structure beneath the invasive and native plants.

In correspondence to the second hypothesis, the comparison between invasive and native plants on soil fungal structure also altered along the urban-periurban gradient. *A. philoxeroides* accumulated more *Russula*, while *T. angustifolia* gathered more *Blumeria* in the urban river. *Russula* is a crucial ectomycorrhizal fungal genera and can form ectomycorrhizal symbiotic relationships with diverse higher plants (Looney et al. 2018). Ectomycorrhizal fungi have been widely considered to extend the plant root ecosystem, aiding in nutrient utilization of soluble and insoluble mineral sources in exchange for carbon allocation from the plant (Landeweert et al. 2001; Looney et al. 2018). *Blumeria* is a plant pathogenic genus causing damage in thousands of plant species (Nowara et al. 2010). Therefore, in the periurban river, the accumulation of *Russula* may foster the expansion of *A. philoxeroides*, while the congregation of *Blumeria* may impede the growth of *T. angustifolia*, which corresponds to the first hypothesis – the invasive plant converges more beneficial microbes relative to the native plant. In the periurban river, *A. philoxeroides* accumulated more *Leucangium* and *Auriculariales*, while *T. angustifolia* accumulated more *Russula. Leucangium* has been identified as an ectomycorrhizal fungal genus as *Russula* while the *Auriculariales* species are saprotrophic (Comandini et al. 2012; Looney et al. 2018; Chen et al. 2021). Since saprotrophic fungi aid in the decomposition of plant litter, the abundance of saprotrophic fungi influence the carbon and nutrient availability of soil (Fontaine et al. 2011). Due to the lack of quantification, we cannot determine if *A. philoxeroides* gathered more beneficial soil fungi compared to *T. angustifolia* in the periurban river, which needs further study.

Conclusion

Using the method of “space over time”, the present study evaluated the impacts of the *A. philoxeroides* invasion on abiotic and biotic constituents – soil stoichiometry and microbial pattern of wetland sediment in a comparison with the native neighbor, *T. angustifolia* within an urbanization gradient. First, we found that the impact of exotic plant invasion on sediment fertility and stoichiometry is overall weak. The invasion of *A. philoxeroides* did not enhance the nutrient availability under most conditions despite a lower soil C/N ratio in the periurban river compared to the native species. A probable explanation for this is the buffering effect of water. Hence, the consideration of water properties is urgent in the future study. Second, we considered that the effects of *A. philoxeroides* invasion on soil microbial diversity are indicator- and context- specific. The invasion of *A. philoxeroides* led to a higher soil bacterial diversity in the periurban river; however, a similar soil bacterial diversity was shared between the two invasive and native plants in the urban river. Additionally, the invasion of *A. philoxeroides* merely resulted in a higher value for Shannon’s diversity index for soil fungi. Third, *A. philoxeroides* may benefit more from soil microbes through shaping a novel microbial structure – accumulation of more soil microbes associated with nutrient cycling, despite the benefit is context-dependent. Future study should certify this prediction using the comparison of plant performance between invasive and native species under respective soil condition. Lastly, we found that urbanization alter the comparison between invasive and native plants on sediment fertility and stoichiometry as well as on soil microbial pattern to a certain extent. We propose

another two future perspectives on this type of study: first, since “the effects on” and “the impacts of” invasion always interwine through the invasion process, the destructure of these two counterparts need to be clarified and thus the interaction between invasive impact and invasion mechanism can be reconciled; second, since the urbanization gradient exists both intra-city and inter-city, a larger picture of the invasion study in urban ecosystem can be drawn within a synergistic application of intra-city and inter-city urbanization gradient.

Declarations

Conflict of interest

The authors declare no conflict of interest.

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Author contribution

T. W. and J. Y. proposed the idea, designed the experiment, analyzed the data, performed the visualization and wrote the manuscript. Y. Z., Z. Z., X. C., Z. S., C. W., L. F., H. D. and Z. F. performed the field investigation, sampling and data collection. C. L. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Figures

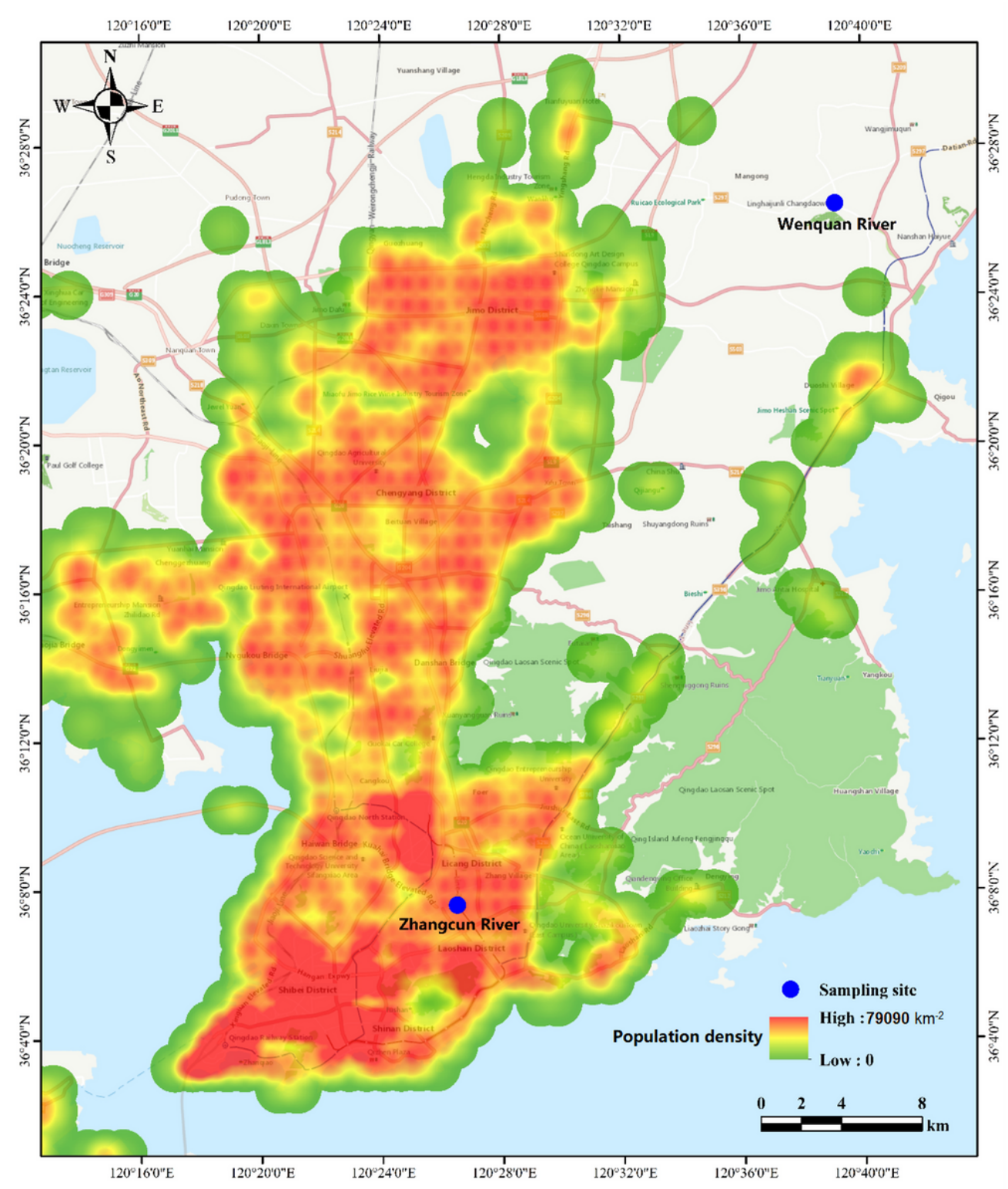

Figure 1

The population density heatmap of two sampling sites – Zhangcun River and Wenquan River

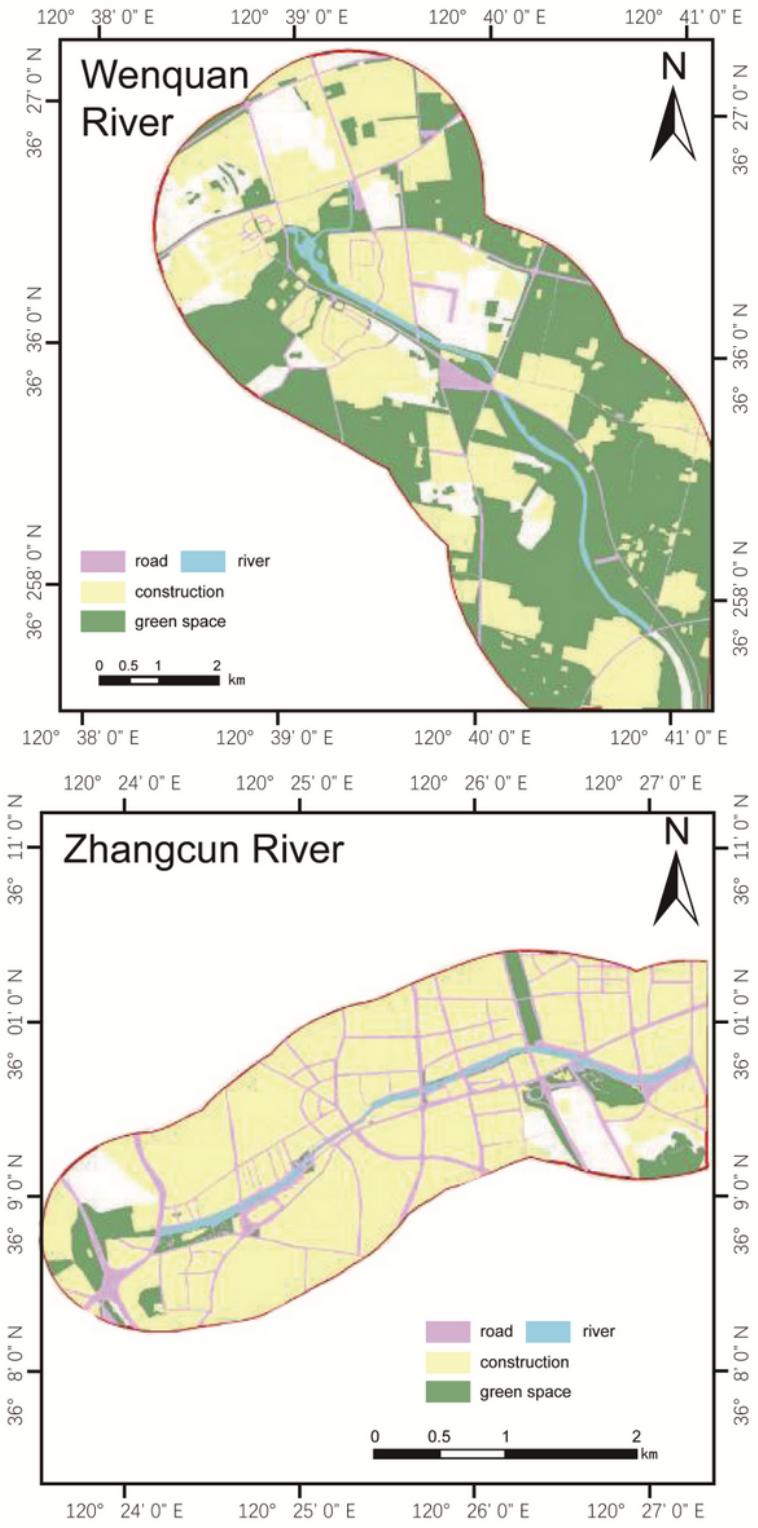

Figure 2

The land use map around the sampling section of two sampling sites – Zhangcun River and Wenquan River. The map shows the areas of impervious surface (roads and constructions) and pervious surface (green space) within 1 km far from the rivers

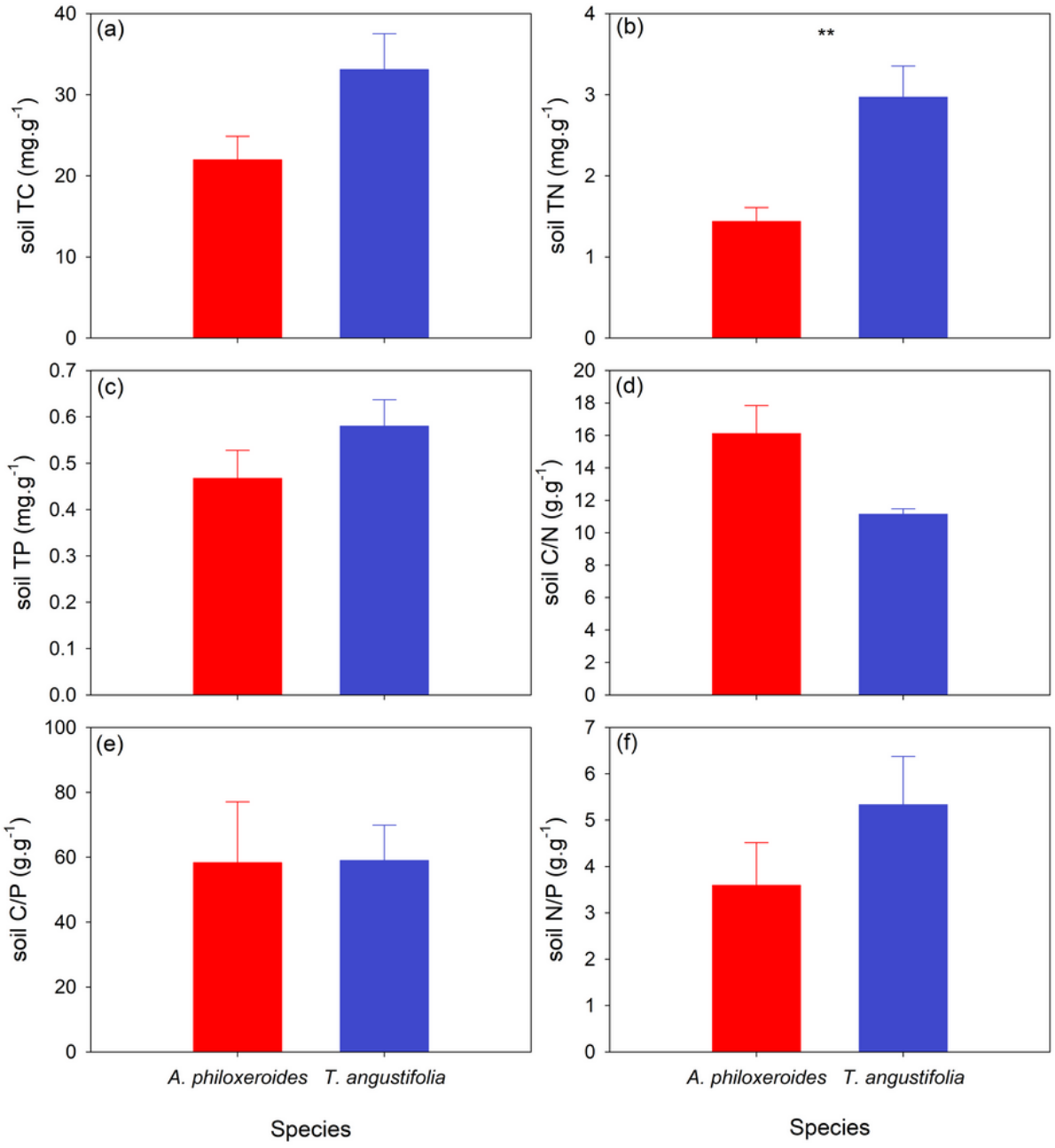

Figure 3

Comparison of soil C, N and P stoichiometry derived from *A. philoxeroides and T. angustifolia* in Zhangcun River (urban). "**" represents "p<0.01"

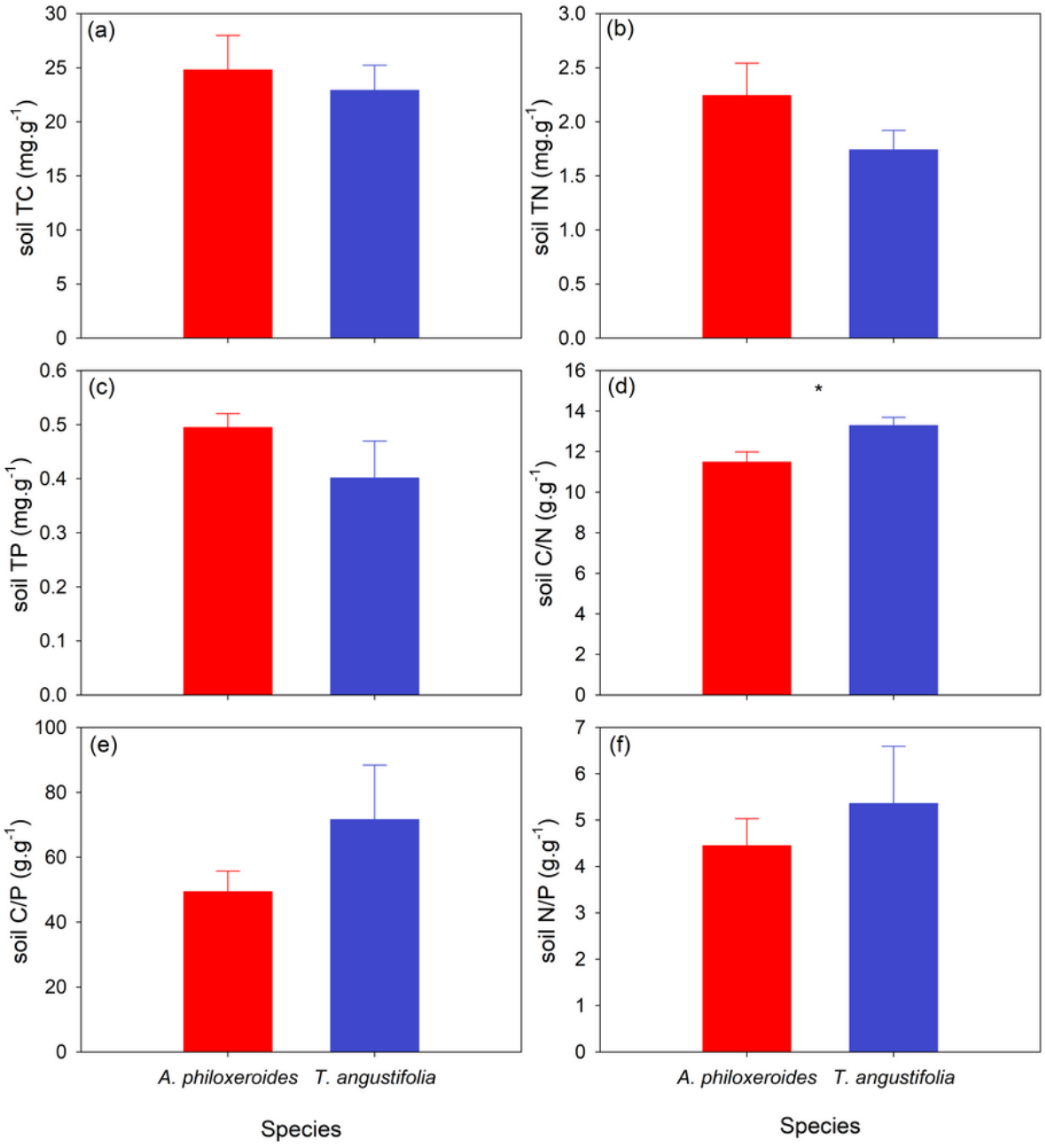

Figure 4

Comparison of soil C, N and P stoichiometry of soils derived from *A. philoxeroides* and *T. angustifolia* in Wenquan River (periurban). "*" represents "p<0.05"

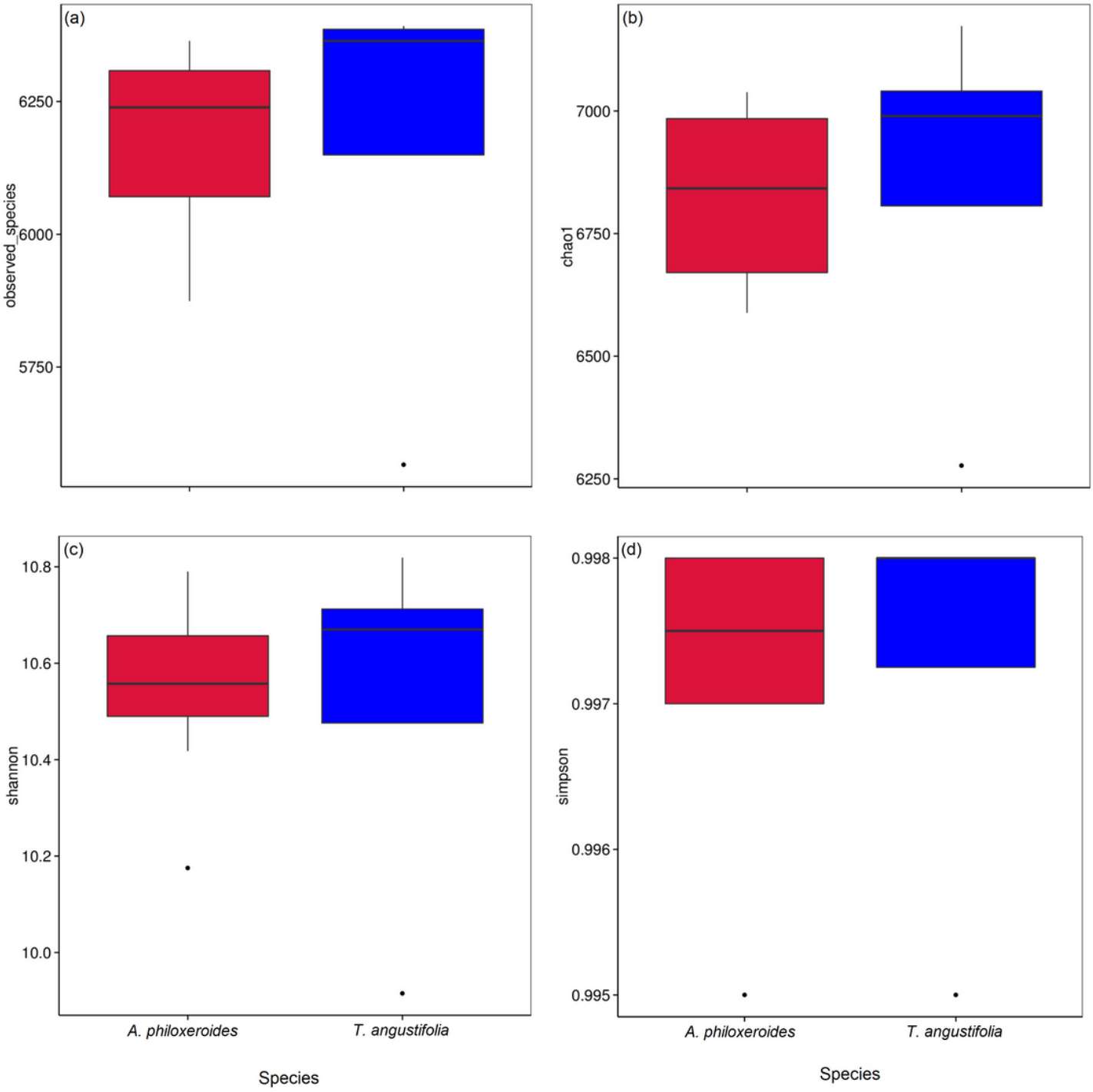

Figure 5

Comparison of soil bacterial α-diversity indexes including (a) observed species, (b) chao1 (c) Shannon's diversity index (shannon) and (d) Simpson's diversity index (simpson) underneath the invasive *A. philoxeroides* and the native *T. angustifolia* in Zhangcun River (urban).

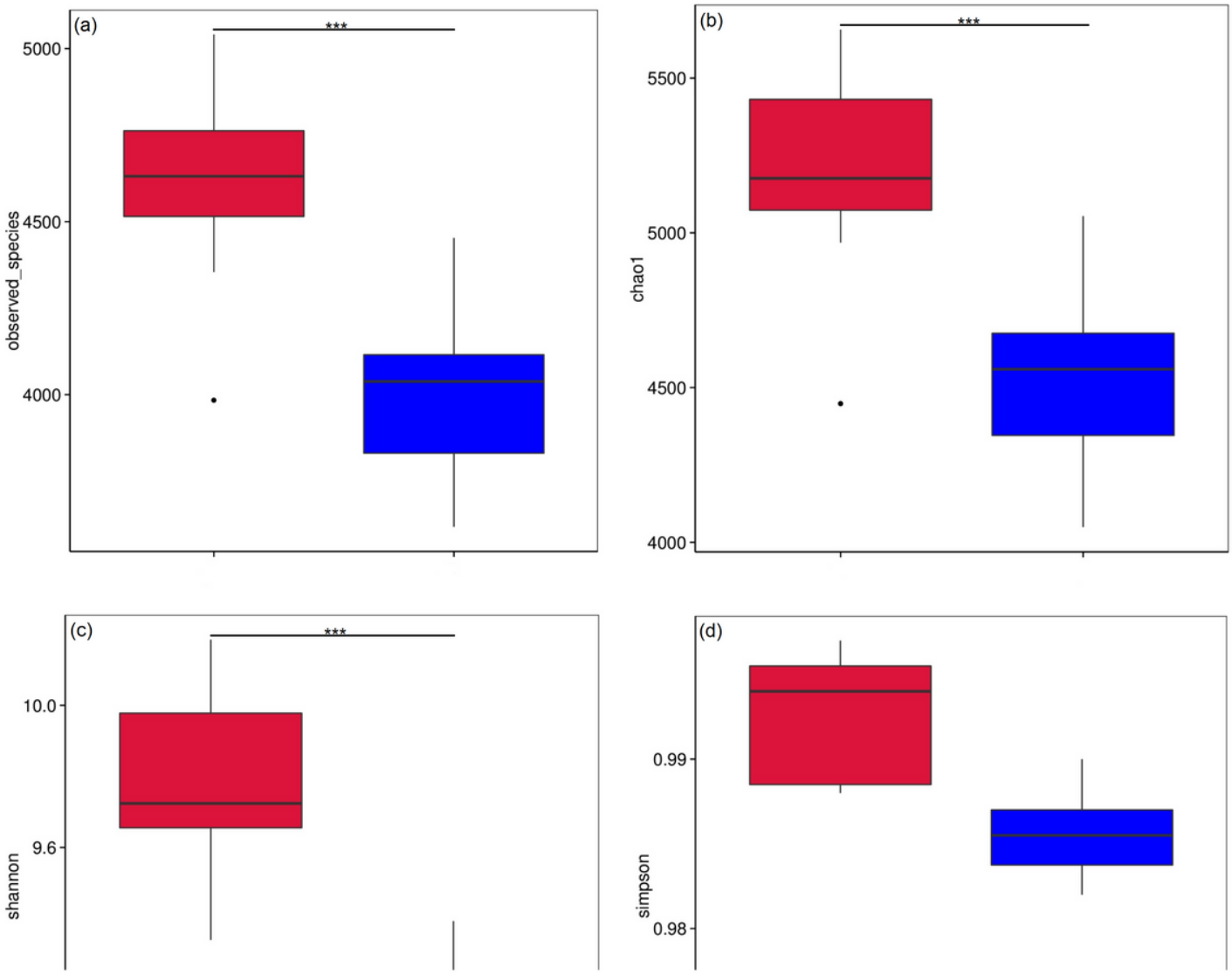

Figure 6

Comparison of soil bacterial α-diversity indexes including (a) observed species, (b) chao1 (c) Shannon's diversity index (shannon) and (d) Simpson's diversity index (simpson) underneath the invasive *A. philoxeroides* and the native *T. angustifolia* in Wenquan River (periurban). "***" represents "*P*<0.001"

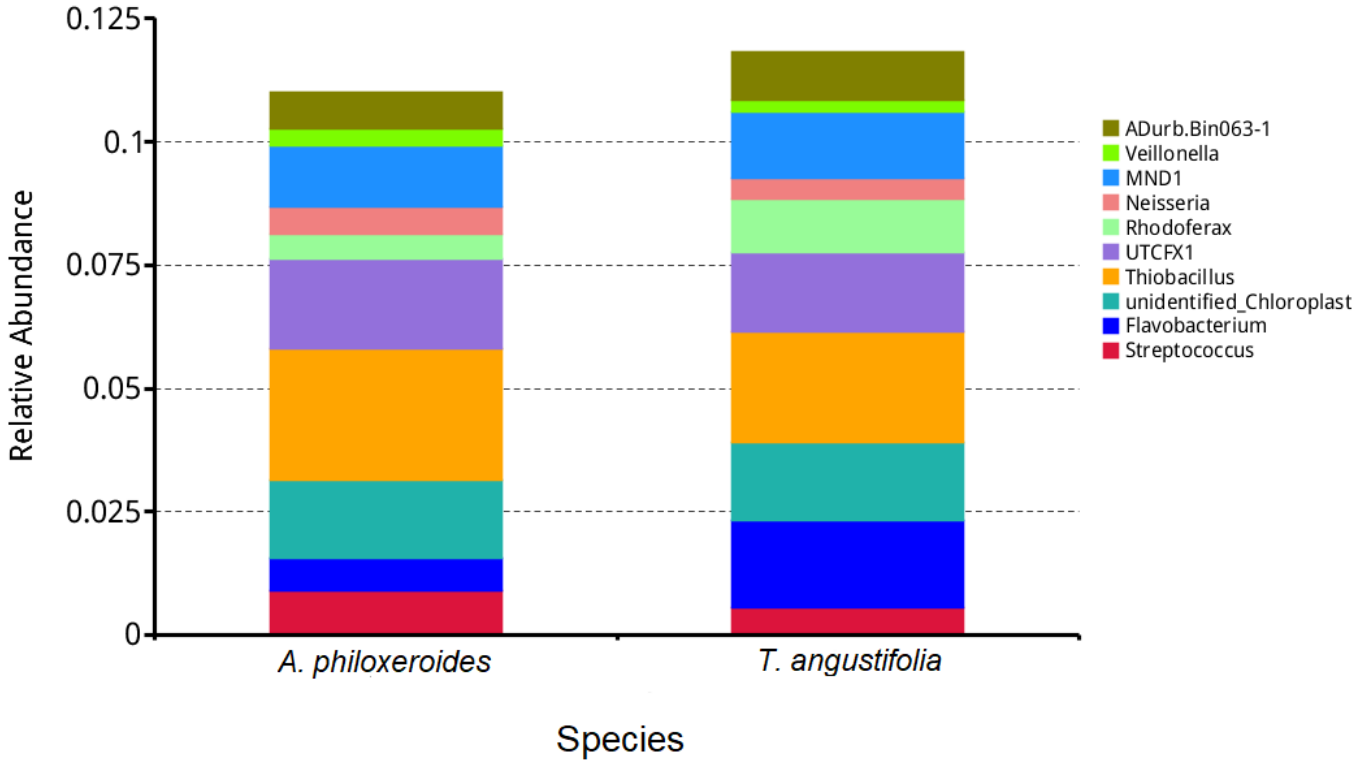

Figure 7

Relative abundance of top 10 bacterial genera underneath the invasive *A. philoxeroides* and the native *T. angustifolia* in Zhangcun River (urban).

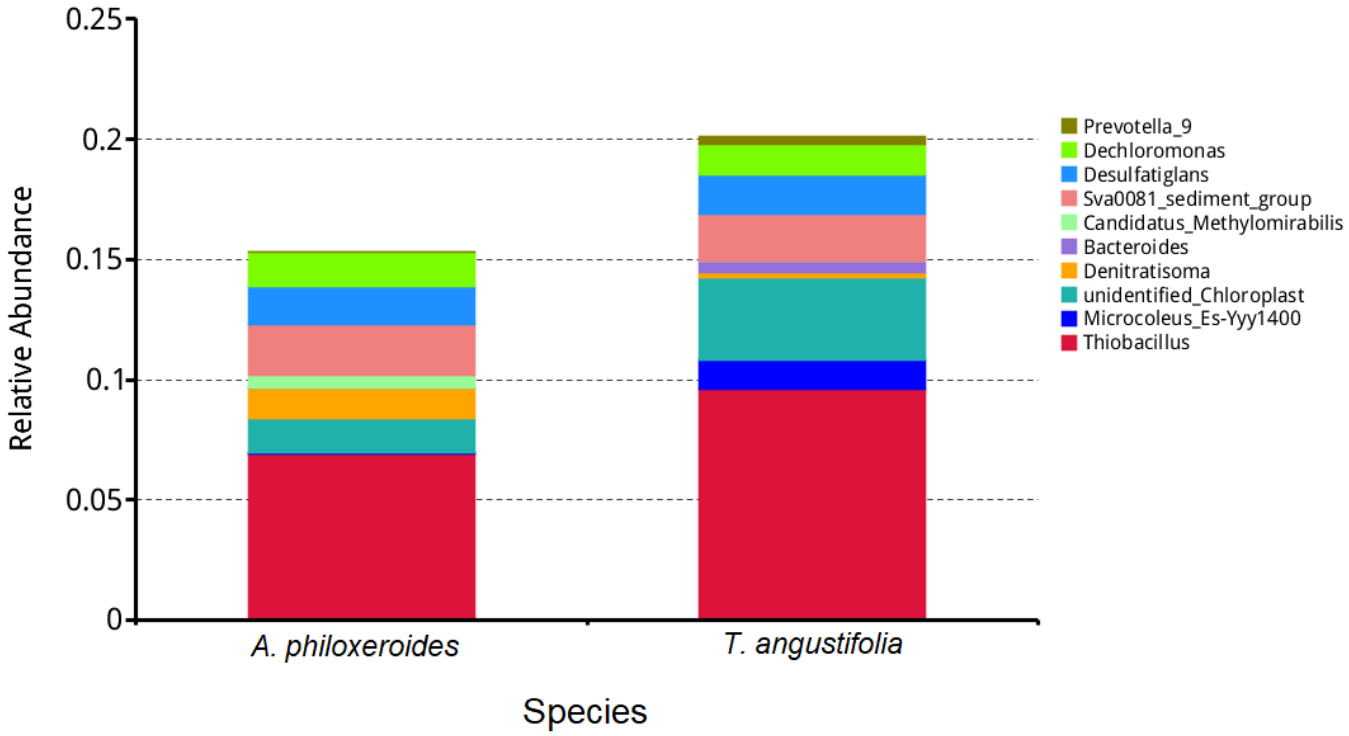

Figure 8

Relative abundance of top 10 bacterial genera underneath the invasive *A. philoxeroides* and the native *T. angustifolia* in Wenquan River (periurban).

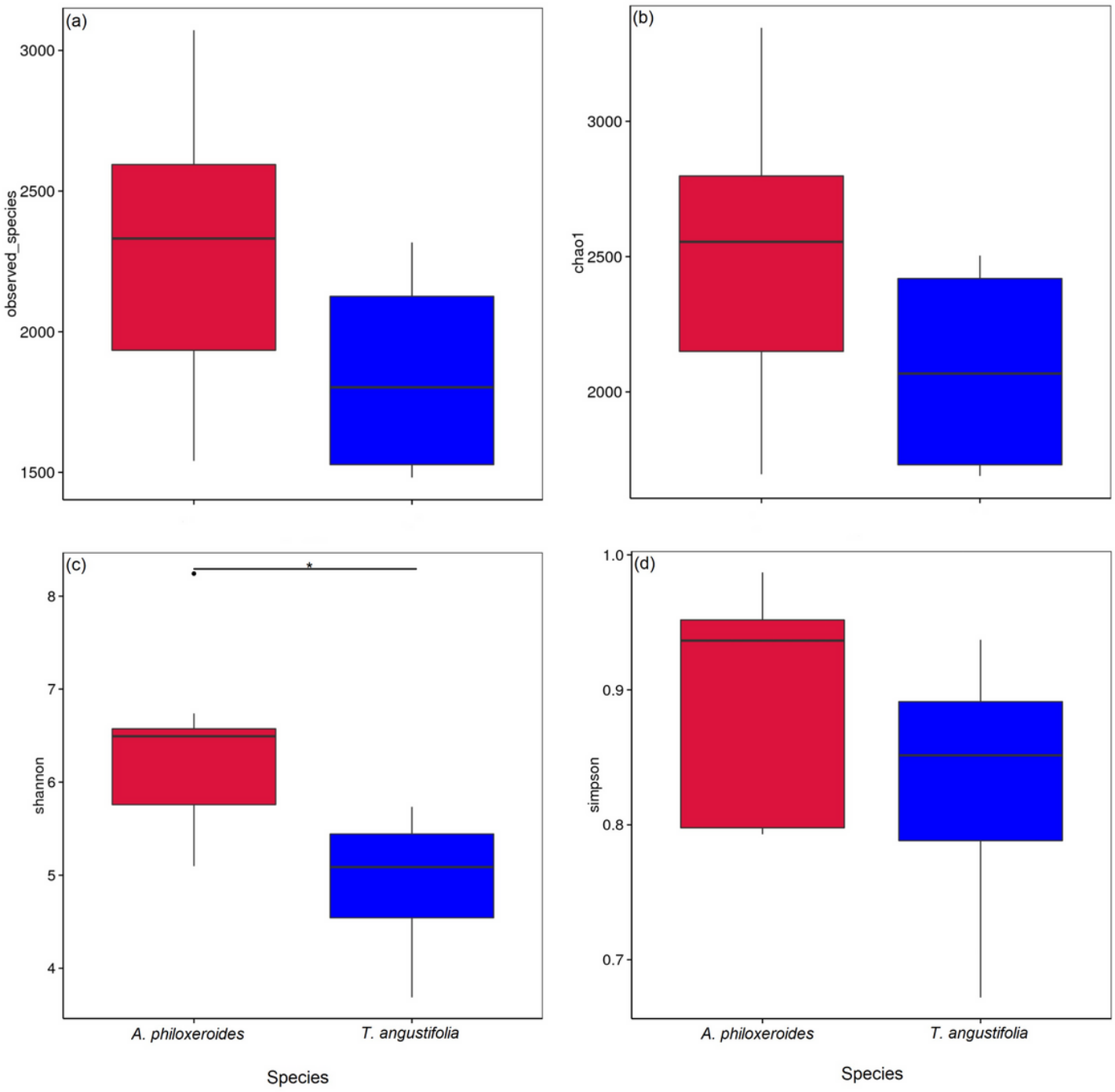

Figure 9

Comparison of soil fungal α-diversity indexes including (a) observed species, (b) chao1 (c) Shannon's diversity index (shannon) and (d) Simpson's diversity index (simpson) underneath different aquatic plant species in Zhangcun River (urban). "*" represents "P<0.05"

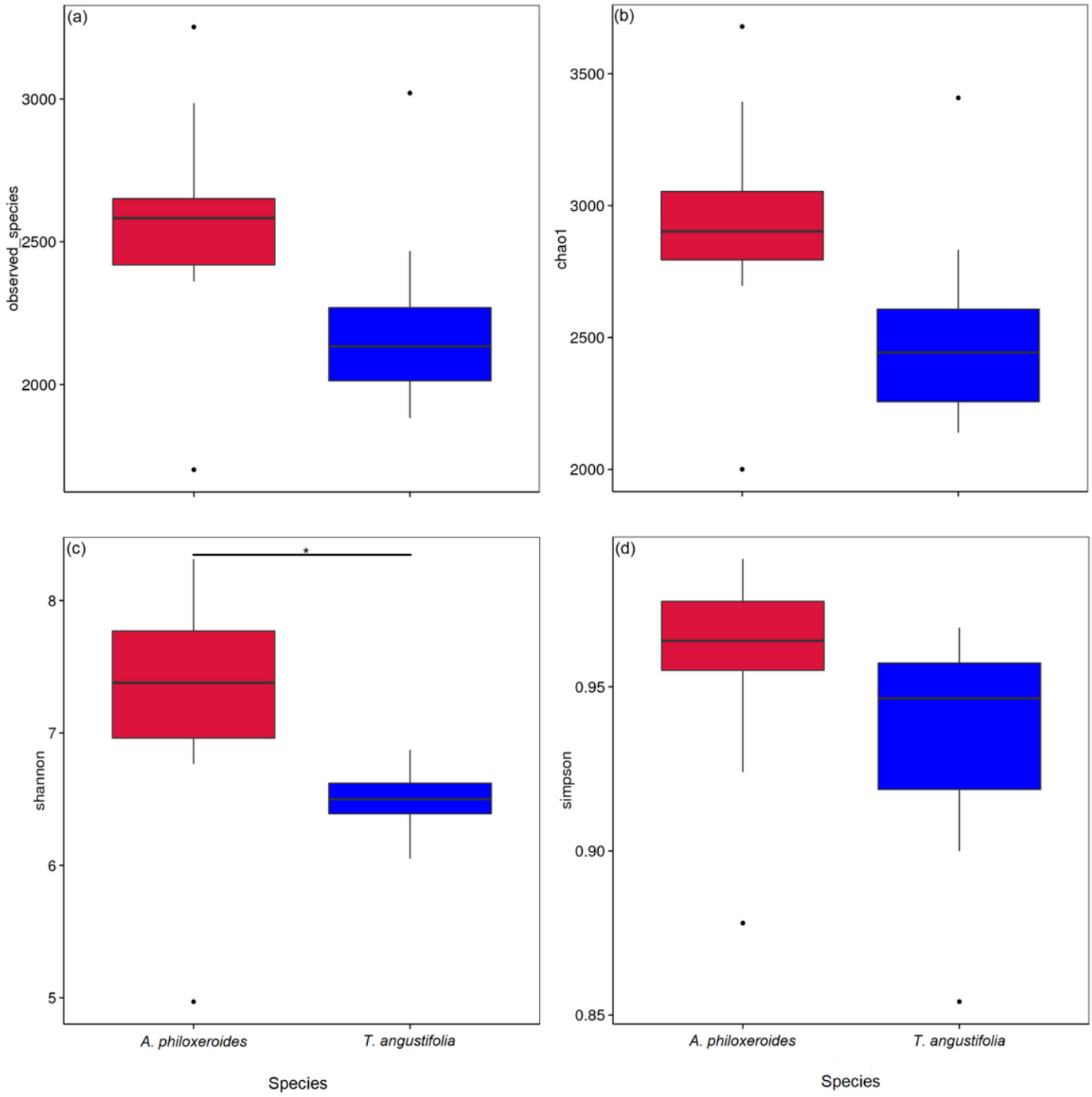

Figure 10

Comparison of soil fungal α-diversity indexes including (a) observed species, (b) chao1 (c) Shannon's diversity index (shannon) and (d) Simpson's diversity index (simpson) underneath the invasive *A. philoxeroides* and the native *T. angustifolia* in Wenquan River (periurban). "*" represents "P<0.05"

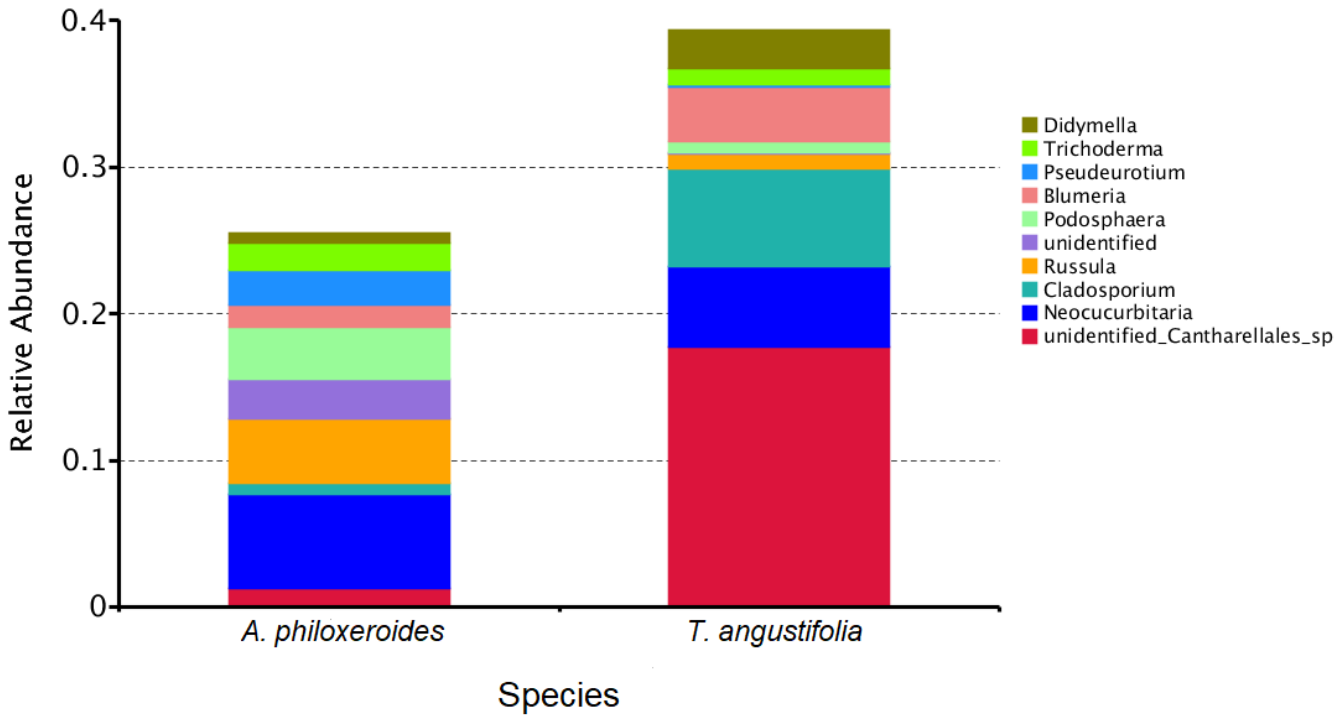

Figure 11

Relative abundance of top 10 fungal genera underneath the invasive *A. philoxeroides* and the native *T. angustifolia* in Zhangcun River (urban).

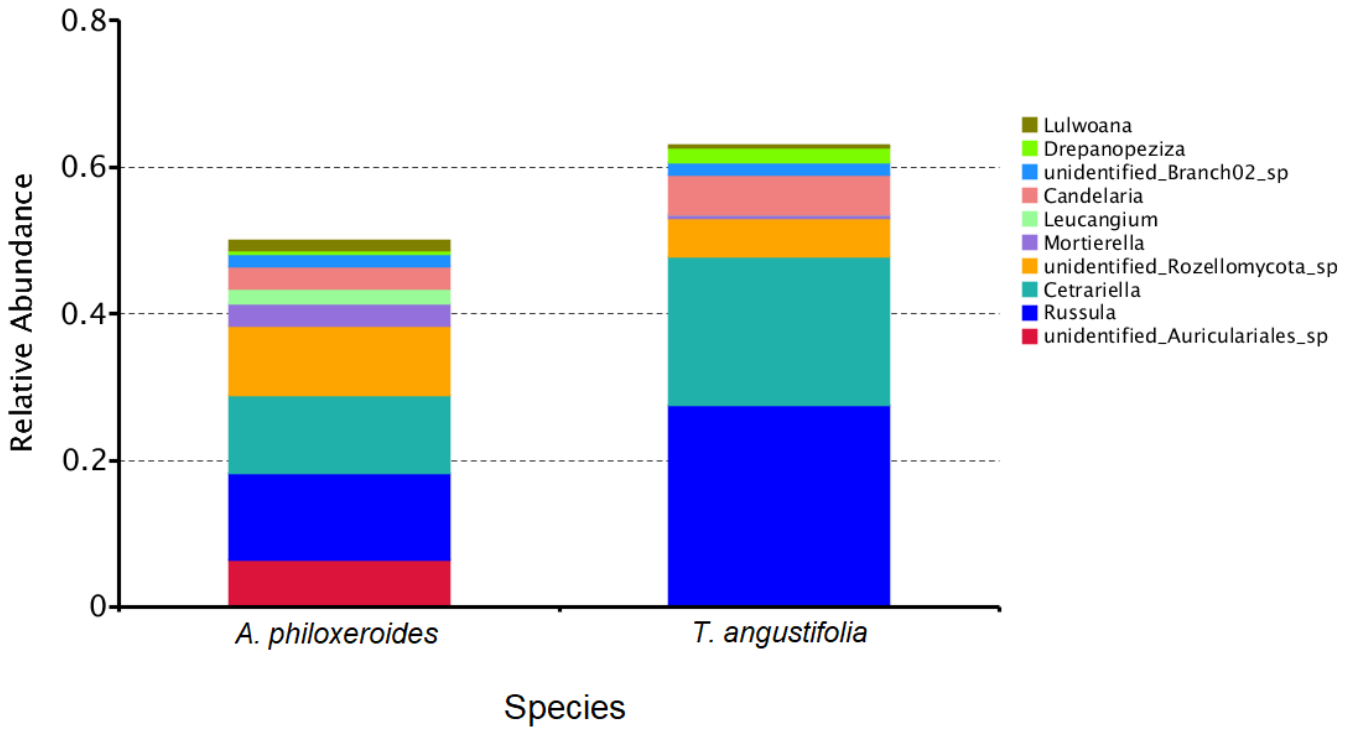

Figure 12

Relative abundance of top 10 fungal genera underneath the invasive *A. philoxeroides* and the native *T. angustifolia* in Wenquan River (periurban).