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Beyond the audible: the effects of broadband noise pollution on the development of an herbaceous and a woody plant

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Although plants and microorganisms can respond to sound (mainly single frequency bands), the effects of anthropogenic noise—particularly broadband traffic noise—remain largely unexplored, especially when plants and soil microbial communities are considered as an integrated system. This study investigates the effects of broadband, traffic-like noise on an herbaceous plant (*Trifolium pratense*) and a woody species (*Ulmus minor*), and on its associated soil bacterial communities to improve understanding of noise-related disturbance in urban and roadside ecosystems. A manipulative experiment was therefore conducted in controlled conditions (phytotron chamber), exposing seeds and plants of the two species to broadband noise emissions following a fully orthogonal experimental design, including a control and a treatment (n = 600 seeds). The main morpho-functional traits were then estimated for both species through time. Lastly, the quali-quantitative structure of soil bacterial communities was also assessed. The results showed that broadband noise induced species- and functional type–dependent responses: negative effects were observed in the woody species, whereas the herbaceous species showed neutral to positive responses. No significant effects on soil bacterial communities were detected. Greater sensitivity of woody species may reflect slower growth rates, longer lifespans, and greater structural and physiological complexity, which can limit rapid compensatory responses, particularly during early developmental stages. These findings identify urban broadband noise as a context-dependent stressor with potential implications for roadside vegetation management, urban plant community resilience, and ecosystem functioning in anthropogenic landscapes.

KEYWORDS
anthropogenic noise, microbiome, noise pollution, plants, urban

1 Introduction

Anthropogenic activities, particularly traffic and industrial operations, generate a wide range of sounds that are increasingly recognized as a major source of noise pollution, affecting biodiversity and ecosystem conservation (Lengagne, 2008; Mason et al., 2016). While 'sound' refers broadly to mechanical vibrations propagating through a medium, 'noise' is herein defined as unwanted or harmful sound, like traffic noise (Fink, 2024).

Accordingly, noise pollution refers to the increase in natural ambient noise levels caused by sound-generating human activities, with potentially detrimental consequences for both humans and wildlife (Slabbekoorn, 2019). Anthropogenic noise is now recognized as pervasive across both natural and urban environments and has been shown to impact a wide range of organisms (Gomes et al., 2021). In fact, its effects on animals have been extensively documented (e.g., Sordello et al., 2020; Bernath-Plaisted and Koper, 2016; Shannon et al., 2016; Drolet et al., 2016; Berkhout et al., 2023; Zaffaroni-Caorsi et al., 2023).

Over the past decades, research has demonstrated that plants are also capable of perceiving and responding to sound stimuli (Kim et al., 2021; De Melo, 2023; Gagliano, 2013), suggesting that they too may be affected by noise pollution. Studies have revealed that different sound frequencies and intensities elicit diverse morphological, physiological, and molecular responses in plants (Mishra et al., 2016; Rodrigo-Moreno et al., 2017; de Paiva Vianna et al., 2015). These include effects on seed germination, growth, oxidative stress, and the expression of stress-related genes (Yi et al., 2003; Cai et al., 2014; Kafash et al., 2022). In this regard, Jung et al. (2018) reported that plants exposed to sound stimuli exhibited altered growth patterns and changes in root development. Additional studies corroborated this hypothesis, showing that prolonged exposure to noise can lead to relevant changes in plant photosynthesis and transpiration rates, ultimately influencing plant growth (Barber et al., 2010). Only a few studies have focused on broadband noise rather than single frequencies and have shown a general worsening in plant health status, as well as a significant decrease in seed germination rates (Lohrey and Talarczyk, 2021). Specifically, according to the available information, anthropogenic sounds seem to influence plant growth rate, biomass production, pigmentation production, and mortality (Shaobin et al., 2010; Gu et al., 2016; Cai et al., 2016; McCauley et al., 2017). Furthermore, the effect of noise can go beyond the direct physiological effects on plants, altering their ecological interactions, including plant–pollinator relationship, and reflecting also on plant associated soil microbial communities (Francis et al., 2012; Robinson et al., 2021), that are known to be strictly related in terms of both abundance and taxa composition to plant species and plant community diversity (Schlatter et al., 2015).

Whereas previous studies have mostly tested the effect of single-frequency or narrow-band sounds kHz on plant response (Huang and Jiang, 2011; Xiujuan et al., 2003; Li et al., 2008; Weinberger and Measures, 1979; Appel and Cocroft, 2014; Cai et al., 2014), very few have investigated the effects of broadband noise (Kafash et al., 2022), which more closely resembles the majority of human-derived sources. Moreover, the impacts of broadband anthropogenic noise on plants and soil microbial communities have rarely been examined simultaneously as an integrated system rather than as separate components. Given the continuous increase of anthropogenic noise in landscapes, it is essential to better characterize its environmental effects. In particular, this pervasive pollutant may influence both plants and soil microbiomes, which play key roles in ecosystem functioning (Guerra et al., 2020). In this context, this study aims to assess the combined responses of plant functional types and soil bacterial communities to broadband, traffic-like noise exposure. To this end, a manipulative experiment was conducted under controlled conditions to assess the responses of urban plant species and the associated soil bacterial communities to broadband noise (specifically pink noise, used as a proxy for traffic noise due to its higher energy at low frequencies (Zambon et al., 2017a; 2017b). The study focused on one herbaceous and one woody species to test the hypothesis that plant life history and functional type mediate noise-induced responses in both plants and the microorganisms inhabiting soils with different vegetation types.

2 Materials and methods

2.1 Considered species

Two plant species with contrasting functional types were selected for the experiment: the herbaceous species *Trifolium pratense* L. and the woody species *Ulmus minor* L. These species are both widespread but differ markedly in their functional traits and, consequently, in their responses to environmental stress (Fermaniuk, 2020). *Trifolium pratense* L. commonly known as red clover, is a temperate perennial herb that grows wild in meadows across Europe and Asia (Zgórka and Maciejewska-Turska, 2021). This leguminous species is widely used in agriculture and is known for its ability to grow under a broad range of environmental conditions, due to its high adaptation potential (Primorac et al., 2007; Taylor and Smith, 1980; Primorac et al., 2007). *Ulmus minor* L., in contrast, is a woody species extensively planted in both rural and urban environments throughout much of Europe, where it can also occur as a spontaneous tree depending on the context, therefore presenting a relatively high tolerance to adverse environmental conditions and stress (Conde et al., 2008; Martín et al., 2019).

2.2 Experimental design

To test the effect of anthropogenic noise on both plant species and on the soil microbiome in the presence of such plants, an artificial broadband noise stimulus (pink noise) was generated using Audacity software. This type of noise was selected for its reproducibility and on sound characteristics, comparable to anthropogenic noise pollution, particularly urban road traffic, where sound energy is typically concentrated at lower frequencies (Zambon et al., 2017a, 2017b).

The experiment was conducted in 2023 in two dedicated phytotrons (Sanyo) with dimensions 180 × 80 × 55 cm (H × L × W): (i) a control; and (ii) a noise treatment one, where a diffuse acoustic field was simulated. Both treatments were considered for each species, according to a fully multi-factorial experimental design, considering both the noise treatment and the species as fixed factors, according to Newman et al. (1997). Each phytotron contained three horizontal shelves, dividing the chamber into four vertical compartments. The loudspeaker was positioned on the first shelf, while plant pots were placed on the second and third shelves (three pots per shelf; Figure 1). For the noise treatment, a speaker (JBL Control 1 Pro) was placed at the center of the top shelf

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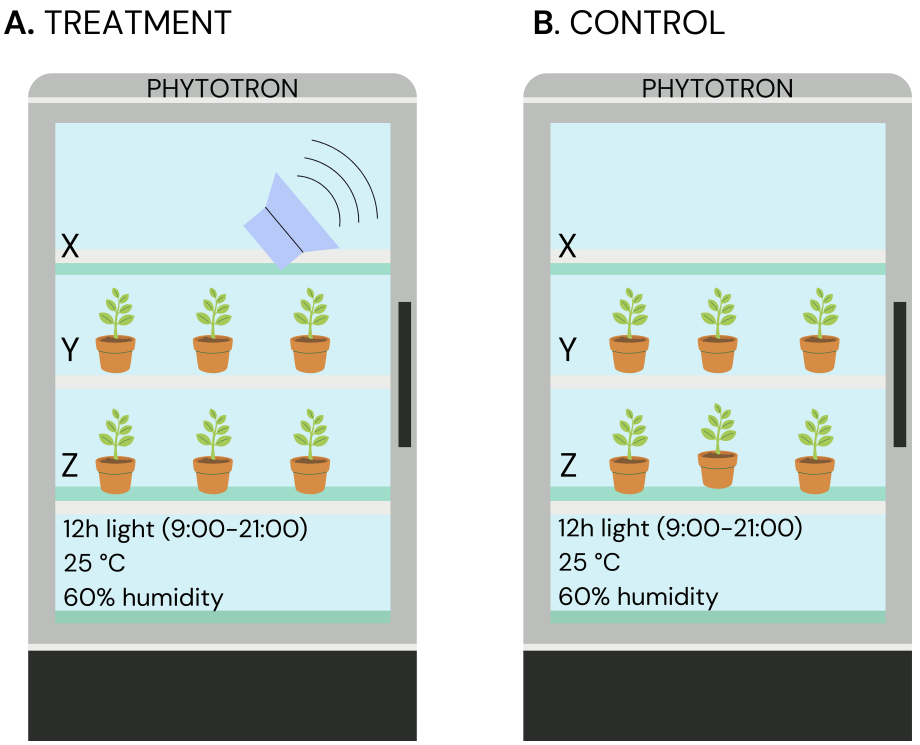

FIGURE 1
Schematic of the experimental design: treatment phytotron **(A)** with a noise signal emitted 24h per day; and control phytotron **(B)** with no noise. X, Y and Z are the designated shelves for the speaker (X) and the vases with seeds.

(designated as shelf X) and oriented toward the rear-right corner. Broadband noise was emitted continuously for 24 hours. Due to technical constraints in reproducing stable long-duration audio with realistic fine-scale temporal fluctuations, noise exposure was applied continuously at a representative average level of a very busy road to ensure experimental consistency, with the study focusing on the effects of noise presence versus absence rather than temporal variability. Consequently, plants were exposed to a constant noise regime to ensure standardized conditions and maximize exposure duration within the experimental constraints. In both phytotrons, identical environmental settings were applied to simulate optimal growth conditions for the selected species. A 12 h light/12 h dark photoperiod was maintained, with light provided from 09:00 to 21:00 and darkness from 21:00 to 09:00, at a constant temperature of 25 °C and a humidity of about the 60%. This photoperiod was designed to mimic natural light–dark alternation and to align with the plants' circadian rhythms. These chambers are intended to regulate microclimatic characteristics and maintain stable conditions in accordance with the set parameters. The seeds of *T. pratense and U. minor* used in this study were certified by Centro di Ricerca, Difesa e Certificazione di Milano and the Regione Carabinieri Forestale Veneto. For its preparation, they were sown in 24 soil-filled pots with a diameter of 14 cm (12 pots per species). The growing medium was prepared by mixing sand and universal potting soil in equal parts (50% each). The commercial potting soil (Vigor Plant complete potting soil) contained 23% coconut fiber, 27% Irish peat, 14% volcanic pumice, and 36% superfine peat, along with a slow-release fertilizer providing nitrogen, phosphorus, and potassium. In each pot, 50 seeds of one of the two species were distributed. For each treatment, six replicated pots (total of 300 seeds), considered as the experimental units, were prepared for each species (N = 12 pots and 600 seeds).

The experiment lasted 42 days (6 weeks), during which the pots were monitored daily, and data on functional traits of both species were collected twice per week, resulting in 12 sampling time points (T1-T12, two per week over six weeks). At the end of the experiment, plants from three randomly selected pots per treatment were harvested for dry weight measurement. Additionally, soil samples were collected from three randomly selected pots per treatment for the quantitative and qualitative characterization of the bacterial communities.

2.3 Phytotron acoustic calibration

Acoustic data were collected using a calibrated Sound Level Meter (SLM) Class 1 (*Larson Davis - 831C*) equipped with a cabled microphone that allowed the placement of the microphone inside the phytotron while keeping the SLM outside for monitoring and data acquisition. To characterize the noise arriving at the plants (LeqZ [dB]), both the background and treatment levels were measured. Mean noise levels in the treatment phytotron were 85.5 ± 1.3 dB, compared with 79.5 ± 2.2 dB in the control. Treatment levels were calibrated to approximate traffic noise at road margins (McAlexander et al., 2015; Wilk et al., 2025) while accounting for the elevated baseline noise generated by the temperature-control ventilation system, which was necessary to ensure precise regulation of environmental conditions. The treatment exceeded this baseline by ~6 dB (≈fourfold increase in sound

intensity) and differed in spectral composition, with the pink noise focusing on higher levels of low frequency sounds, supporting the attribution of observed effects to traffic noise exposure rather than background noise.

To ensure uniform noise exposure among plants, pots were regularly repositioned within each phytotron throughout the experiment, as spatial variation in sound levels within phytotron chambers has been previously reported (Zaffaroni-Caorsi et al., 2025). A detailed acoustic characterization of the phytotrons, including spatial consistency under both light-on and light-off conditions, is reported in Zaffaroni-Caorsi et al. (2025).

2.4 Analysis of plant morpho-functional traits

The following morpho-functional traits were measured for both species: total and through-time emergence, survival, growth, and health status. Specifically, emergence dynamics were analyzed by considering the emerged seedlings throughout the experiment (at all 12 sampling points, T1–T12), while total emergence (%) was collected at the end of the experiment (T12). Survival was expressed as the number of plants per pot at the end of the experiment (T12). Growth was assessed considering the plant height (cm), the leaf number, the leaf area (cm^2), and the total dry biomass (hypogeal + epigeal dry weight (g); at the end of the experiment (T12). Finally, plant health status was evaluated by measuring photosynthetic efficiency (Fv/Fm), using a Plant Efficiency Analyzer at the end of the experiment (T12).

2.5 Quantitative characterization of the bacterial community

A bacterial suspension was prepared from the soil (1 g) collected randomly from three of the six available pots for each treatment and species (N = 3), in order to guarantee statistical relevance of the obtained results, considering that, according to literature, three replicates are sufficient to characterize in detail bacterial communities in terms of abundance and also taxa composition (*e.g.* Caronni et al., 2025, 2023). The suspension was prepared following Barbesti et al. (2000), separating bacteria from soil using a sterilized 10 mM MgSO4 solution (10mL of MgSO4 for each g of soil). SYBR Green (1:10000 dilution) and propidium iodide (1 mg/1 mL) fluorochromes were used for staining, counting separately live and dead bacteria. Bacterial abundance was expressed as the number of bacteria per gram of soil.

2.6 Qualitative characterization of the bacterial community

Bacterial DNA was extracted from the soil (500 mg) of three random pots for each treatment and species (N = 3), using the FastDNA® Spin for Soil kit (MP Biomedicals, Solon, OH, USA), according to the manufacturer's instructions (FastDNA SPIN Kit for Soil, User Manual). After a first PCR amplification (using 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 519R (5'-GWATTACCGCGGCKGCTG-3') primers for each sample, to assess the DNA sample quality, a second PCR was performed targeting the V5–V6 hypervariable regions of the 16S rRNA gene, using GoTaq® Green Master Mix (Promega Corporation, Madison, WI, USA). Customized 6 bp oligonucleotide barcodes were added to the 5′ end of the primers 783F and 1046R (Huber et al., 2007).

The amplicons were then purified with the Wizard® SV Gel and PCR Clean-up System kit (Promega Corporation, Madison, WI) and quantified with the Qubit® 2.0 fluorometer (Life Technologies, Carlsbad, CA). To perform Illumina sequencing, libraries containing 9 samples (with specific barcodes) each were prepared. Standard adapters called Nextera indexes (Illumina, Inc., San Diego, CA) were used to prepare the libraries. Sequencing was then performed with the Illumina MiSeq platform, using a 2 x 300 bp paired-end protocol.

The obtained reads were demultiplexed according to the indexes and barcodes. The Uparse pipeline was used for the following elaborations. Forward and reverse reads were merged only if there were zero mismatches, and quality filtered with default parameters. The sequences were then analyzed using ASVs (Amplicon Sequence Variants), which represent groups of identical sequences (with a 100% degree of similarity). The DADA2 algorithm was used to identify the ASVs, correcting or eliminating those considered incorrect due to PCR errors. The ASVs were created, filtered, and then classified by comparing them with the RDP and SILVA databases (McAlexander et al., 2015; Wilk et al., 2025).

2.7 Statistical analysis

Both univariate and multivariate statistical analyses were applied to process the data. Univariate analyses, including analyses of variance (ANOVA) and Student-Newman-Keuls (SNK) *post hoc* tests, were conducted using GMAV5 software to assess differences in morpho-functional traits between the two species and the bacterial abundances. Multivariate analyses were conducted using PERMANOVA in PrimerV7 + PERMANOVA software to evaluate differences in the taxonomic composition of the bacterial communities. A detailed description of the statistical procedures is provided hereafter.

With regard to univariate analyses, for emergence through time, due to data dependence between sampling times, it was not possible to use the ANOVA test to analyze data (Underwood, 1997). Therefore, dependent sample t-tests were used to compare data obtained at different sampling times (T2 *vs* T4 *vs* T6 *vs* T8 *vs* T10 *vs* T12). For the other traits, ANOVA was used. Specifically, Cochran's tests were run prior to each ANOVA to test for homogeneity of variances and normality was assured by Kolmogorov-Smirnov test. Student–Newman–Keuls (SNK) tests were used for a posteriori comparison in case of significant ANOVA results, testing all possible pairs of means computed across the considered groups (Underwood, 1997).

Specifically, for some of the considered morpho-functional traits (total emergence, survival, photosynthetic efficiency), 3 two-way ANOVAs were run, one for each response variable, to test for differences between the two species (Species, 2 levels, fixed: *T.*

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pratense vs *U. minor*) and the two treatments (Noise, 2 levels, fixed: *Noise* vs Control) at the end of the experiment (T12). For other traits (plant height, number and area of leaves, and dry weight), data regarding the two species were related to their particular features and phenology and were therefore not comparable. For this reason, different 3 one-way ANOVAs were run in this case, one for each species for each response variable, to test for differences between the two treatments (Noise, 2 levels, fixed: *Noise* vs Control) for each species at the end of the experiment (T12). Finally, with regard to bacterial abundance, a 1 two-way ANOVA was run to test for differences between the two species (Species, 2 levels, fixed: *T. pratense* vs *U. minor*) and the two treatments (Noise, 2 levels, fixed: *Noise* vs Control) at the end of the experiment (T12). Moreover, the phytotron was considered a blocking factor in the statistical model to account for potential between-chamber confounding effects.

With regard to multivariate analyses, they were based on the Hellinger distance, which depends on differences in ASV proportions between samples, decreases the importance of ASV abundance over their occurrence, and avoids the double-zero problem when comparing ASV composition between samples (Legendre and Legendre, 2012; De Cáceres et al., 2010). Non-metric multidimensional scaling (nMDS ordination) was performed on all samples to visualize the data distribution. Moreover, a distance-based permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) was then performed to test for differences in the genus composition of the bacterial community in relation to the species (Species, 2 levels, fixed: *T. pratense* vs *U. minor*) and the treatment (Noise, 2 levels, fixed: Noise vs Control) (PERMAnova). The analyses were based on Bray-Curtis dissimilarities calculated on normalized data (Hellinger). Each term in the analysis was tested using 9999 random permutations, considering as permutation method the unrestricted permutation of raw data.

3 Results

3.1 Trifolium pratense and Ulmus minor functional traits

Considering the temporal dynamics of emergence, exposure to noise resulted in a delay in germination for *U. minor*, whereas this effect was not observed for *T. pratense*. Specifically, for *U. minor*, the woody species, no seedling emergence was recorded during the first week under noise exposure, while several seedlings emerged in the control treatment over the same period (Figure 2A; Table 1).

Total emergence was significantly higher for *T. pratense* in comparison with *U. minor*, under noise exposure (ANOVA, $P<0.05$) (Figure 2B; Table 2). Moreover, no significant differences in mean emergence were observed between treatments (noise vs. control) for *T. pratense*. In contrast, *U. minor*, exhibited a significantly lower number of germinated seedlings under noise exposure compared to the treatment (ANOVA, $P<0.05$).

Regarding plant survival, no differences were detected at the end of the experiment between species or treatments, although *U. minor* showed slightly lower values under noise exposure (Figure 3A; Table 2). Nonetheless, *U. minor* exhibited a significantly lower photosynthesis efficiency under noise exposure (ANOVA, $P<0.05$), whereas no differences between the noise and control treatments were observed for *T. pratense* (Figure 3B; Table 2).

With respect to plant growth, significant differences in the height, as well as in leaf number and leaf area, were observed between treatments for *U. minor*, but not for *T. pretense* (ANOVA, $P<0.05$ for the height and the leaf number; $P<0.01$ for the leaf area) (Figures 4A–C; Table 3). Specifically, for all these traits, lower values were recorded for the former species under noise exposure. Regarding total dry weight, *U. minor* showed significant lower values when exposed to noise, while *T. pratense* showed a reverse

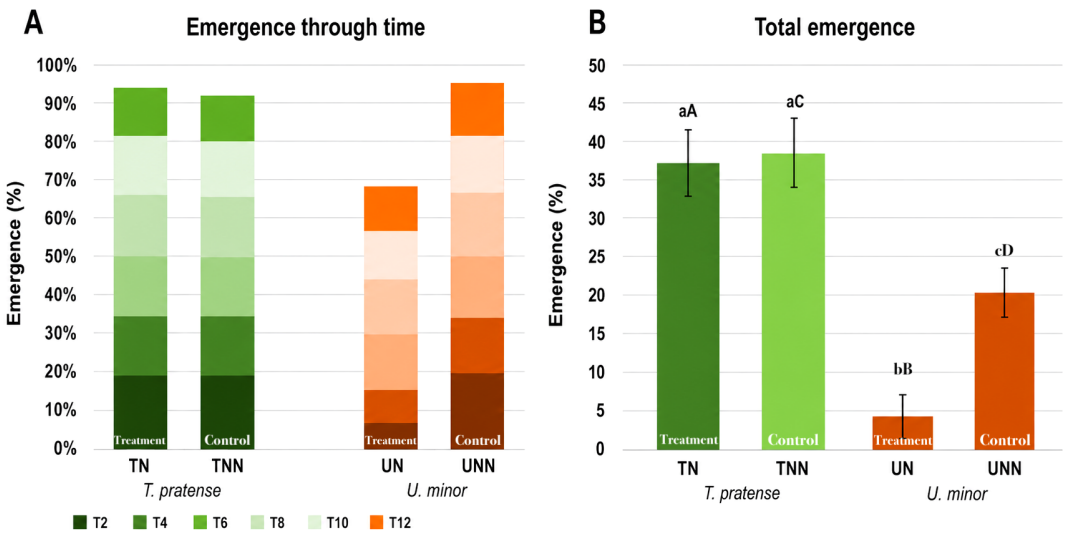

FIGURE 2
Plant emergence: emergence (%) through time **(A)** and mean (+SD) values at the end of the experiment, **(B)** for the two species (T, *T. pretense* (green bars); U, *U. minor* (brown bars)) for the noise (N: dark color) and the control (NN: light color) treatment. For emergence through time, color gradations refer to the different sampling times (T2, T4, T6, T8, T10, T12). For total emergence, different letters indicate significant differences ($P<0.05$). The first lower case letter indicates differences among treatments within species. The second upper case letter refers to differences between species for each treatment.

TABLE 1 Results of dependent sample t-tests used to compare the emergence thought time (T2 vs T4 vs T6 vs T8 vs T10 vs T12) for the two species in relation to noise (N: Noise vs NN: control).

Noise	Species	t	P
T2 vs T4			
N	*T. pratense*	0.45	*0.2132*
NN	*T. pratense*	0.18	*0.4567*
N	*U. minor*	0.67	*0.0007*
NN	*U. minor*	0.89	*0.0003*
T4 vs T6			
N	*T. pratense*	0.12	*0.2134*
NN	*T. pratense*	0.92	*0.4567*
N	*U. minor*	0.76	*0.7894*
NN	*U. minor*	0.91	*0.2134*
T6 vs T8			
N	*T. pratense*	0.56	*0.4567*
NN	*T. pratense*	0.32	*0.9123*
N	*U. minor*	0.82	*0.5673*
NN	*U. minor*	0.12	*0.3212*
T8 vs T10			
N	*T. pratense*	0.23	*0.3412*
NN	*T. pratense*	0.88	*0.5678*
N	*U. minor*	0.47	*0.4568*
NN	*U. minor*	0.43	*0.5123*
T10 vs T12			
N	*T. pratense*	0.65	*0.7235*
NN	*T. pratense*	0.78	*0.8215*
N	*U. minor*	0.78	*0.5125*
NN	*U. minor*	0.26	*0.7345*

condition, with lower dry weights found for the control plants (Figure 4D; Table 3).

3.2 Quantitative and qualitative characterization of soil bacterial communities

No differences in soil bacterial abundance were observed between the two plant species for both treatments, with similar abundances recorded overall (Figure 5; Table 4). Moreover, no significant difference was observed overall in the structure of soil bacterial communities among treatments (Figure 6; Table 5), both at the beginning but especially, also at the end of the experiment. At the end of the experiment, in particular, bacterial abundance was similar between the two applied treatments for both *T. pratense* (550000000 ± 1002356 cells/cm^2) and *U. minor* (570000000 ± 1214792 cells/cm^2). Furthermore, the composition of the considered communities didn't change with noise exposure (Figure 6; Table 5). In all considered conditions, soil bacterial communities were characterized by a very high number of ASVs (1745), independent of the associated plant species and the applied treatment (Figures 6, 7). The taxa composition of the considered bacterial communities is detailed in Figure 7. Specifically, for both the species and the applied treatments, the most abundant bacterial genera were present with similar percentages in all the samples; in particular, *Unclassified_Myxococcales* accounted for approximately 8%, while the genera R*hodanobacter, Ramlibacter* and *Chryseolinea* approximately for 7.5%, 6.0% and 5.5%, respectively (Figure 6; Table 5).

4 Discussion

The obtained results provide new insights into the effects of broadband noise on both plants and bacterial communities of

TABLE 2 Results of the ANOVA tests to compare *T. pratense* and *U. minor* performance in relation to noise; statistically significant results (P<0.05) are given in bold.

Source	DF	Total emergence		Survival		Photosynthetic efficiency	
		F	P	F	P	F	P
Species (Sp)	1	6.721	**0.023**	8.562	0.0123	9.678	0.051
Noise (N)	1	7.812	**0.034**	9.312	0.834	8.924	0.721
Te x N	*2*	5.912	**0.021**	7.567	0.078	8.781	**0.045**
RES	5						
TOT	10						
Cochran's Test	C = 0.9324 *ns*			C = 0.7234 *ns*		C = 0.9213 *ns*	
SNK test (SE = 11.2345)	Species N = T>U NN = T>U NoiseTemperature T= N=NN U= NN>N					*SNK test (SE= 12.7234)*	Species N = T>U NN = T=U Noise T= N=NN U= NN>N

When P<0.05, SNK *post-hoc* test results are also provided.

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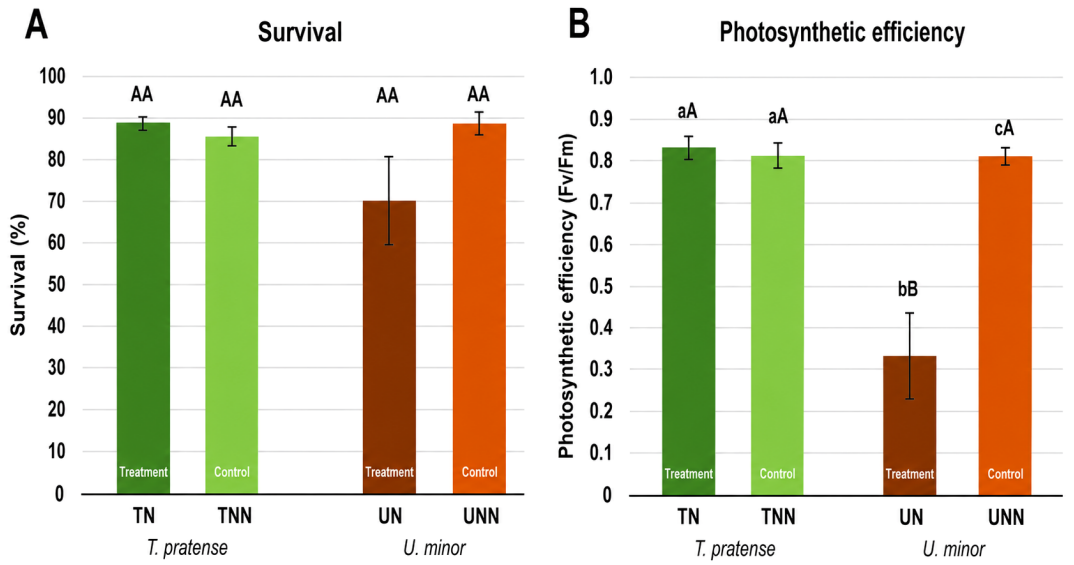

FIGURE 3
Plant survival and photosynthesis: mean (+SD) values for survival (%) **(A)** and photosynthetic efficiency (Fv/Fm) **(B)** for the two species (T, *T. pretense* (green bars); U, *U. minor* (brown bars) in noise (N: dark color) and control (NN: light color) treatments at the end of the experiment. Different letters indicate significant differences (P<0.05). The first lower case letter indicates differences among treatments within species. The second upper case letter refers to differences between species for each treatment.

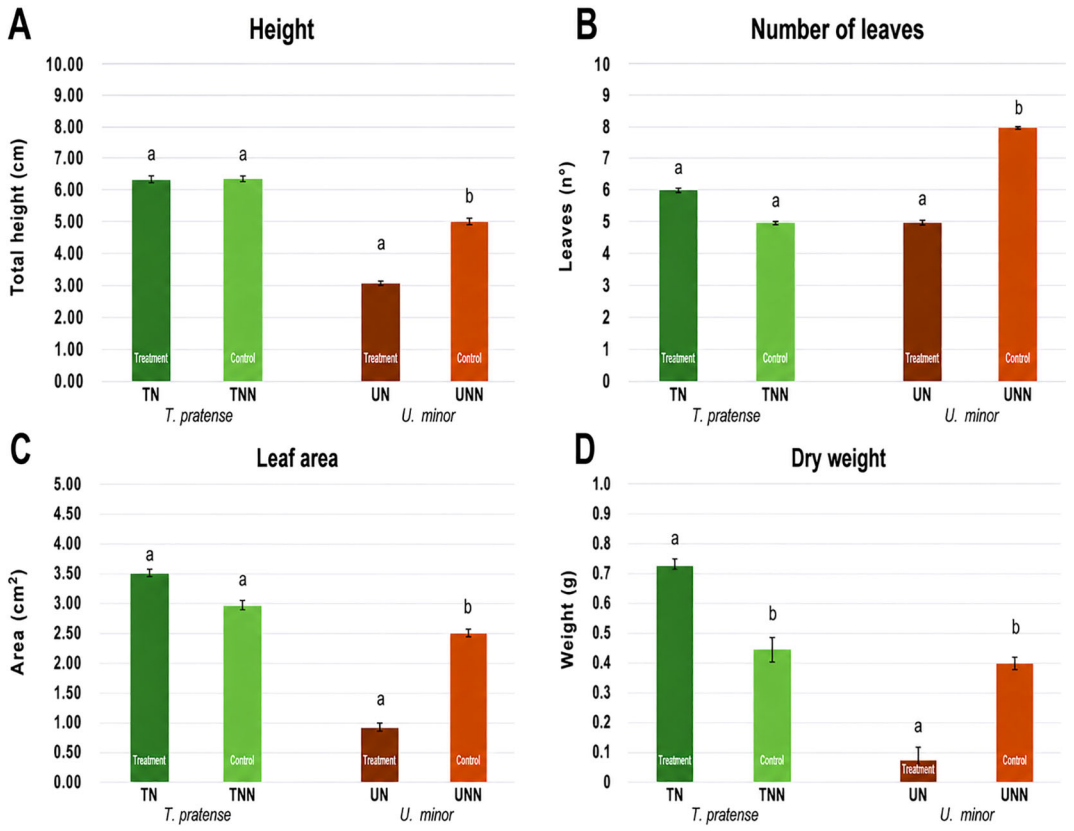

FIGURE 4
Plant growth: mean (+SD) of height **(A)**, number of leaves **(B)**, leaf area **(C)** and dry weight **(D)** for the two species (T, *T. pretense* (green bars); U, *U. minor* (brown bars) in the noise (N: dark color) and control (NN: light color) treatment at the end of the experiment. Different letters indicate significant differences (P<0.05) (and italic is used to distinguish between the different statistical analyses performed (please see the SM section for details).

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TABLE 3 Results of the ANOVA tests to alternatively evaluate *T. pratense* and *U. minor* performance in relation to noise. Statistically significant results (P<0.05) are given in bold.

T. pratense		Height		Number of leaves		Dry weight		Leaf area	
Source	DF	F	P	F	P	F	P	F	P
Noise (N)	1	11.821	0.721	8.462	0.321	7.112	**0.042**	6.779	0.342
RES	3								
TOT	5								
Cochran's Test	C = 0.5345 *ns*			C = 0.9356 *ns*		C = 0.4213 *ns*		C = 0.9134 *ns*	
						SNK test	N>NN		
						(SE = 10.2340)			

U. minor		Height		Number of leaves		Dry weight		Leaf area	
Source	DF	F	P	F	P	F	P	F	P
Noise (N)	1	10.112	**0.021**	9.889	**0.035**	10.512	**0.025**	7.589	**0.01**
RES	3								
TOT	5								
Cochran's Test	C = 0.7213 *ns*			C = 0.8216 *ns*		C = 0.9235 *ns*		C = 0.3567 *ns*	
	SNK test		NN>N	SNK test	NN>N	SNK test	NN>N	SNK test	NN>N
	(SE = 11.114)		(SE = 10.875)	(SE = 11.7234)		(SE = 10.0030)			

When P<0.05, SNK *post-hoc* test results are also provided.

vegetated soils, simulating, in particular, urban traffic noise. Overall, the two considered species, the woody *U. minor* and the herbaceous *T. pratense* exhibited markedly different responses to noise: anthropogenic sound exposure significantly impaired the germination, growth, and photosynthetic efficiency of the woody plant *U. minor*, without affecting its survival. Whereas, the herbaceous species, *T. pratense*, remained largely unaffected by noise, even increasing its biomass production when exposed to the artificial signal. Meanwhile, no significant effect was observed in the bacterial community in the presence of both plants.

4.1 Germination responses

Focusing on germination, *Ulmus minor* experienced a clear delay in emergence when exposed to noise, with almost no seedlings observed during the first week of the experiment. Differences between noise and control conditions were also evident between both species when considering final emergence, indicating that noise significantly affected early developmental stages on the woody plant. These results are consistent with the few studies available on this topic (Zhou et al., 2021; Pérez-Harguindeguy et al., 2013),

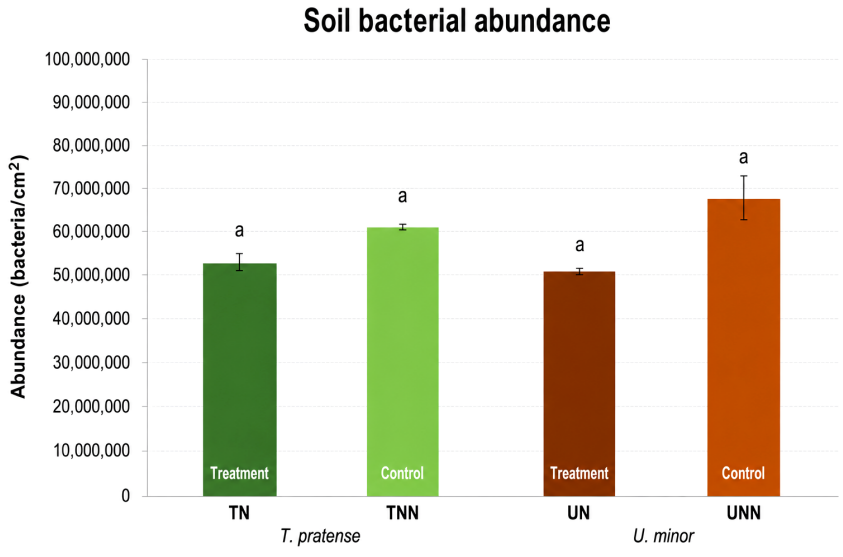

FIGURE 5
Soil bacterial abundance (mean +SD) for the two species (T: *T. pretense* (green bars); U: *U. minor* (brown bars)) in noise (N: dark color) and control (NN: light color) treatments at the end of the experiment. Different letters indicate significant differences (P<0.05).

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TABLE 4 Results of the ANOVA to test for differences in the bacterial abundances between soils with *T. pratense* and *U. minor* plants in relation to noise.

Source	DF	T0	
		F	P
Species (Sp)	1	6.672	0.135
Noise (N)	1	3.421	0.541
Sp x N	*2*	5.668	0.673
RES	5		
TOT	10		
Cochran's Test	C = 0.7891 *ns*		

which suggest that noise interferes with early developmental processes, potentially through alterations in seed metabolism (Gagliano, 2013). In addition to direct effects on seeds, noise may indirectly influence germination by modifying soil microenvironmental conditions (Gagliano, 2013). Previous studies have shown that sound vibrations can affect key physical properties of soils, including water movement through pore systems (Flammer et al., 2001) and, more broadly, the structure of the soil matrix (DiCarlo et al., 2003). Such changes could further constrain germination and emergence, particularly in species requiring stable environmental conditions during early development. On the contrary, some studies have demonstrated that exposure to specific sound frequencies can enhance germination and seedling growth. For instance, Cai et al. (2014) reported faster germination and improved seedling growth in mung beans exposed to 2000 Hz sound at 90 dB compared to those exposed to lower or higher frequencies. These differences might be related to the composition of the sound frequencies emitted (Azgomi et al., 2023). In particular, exposure to strong, disharmonious sound vibrations, commonly associated with noise pollution, has been shown to activate plant defence system, triggering a cascade of biological responses. Such effects may be especially pronounced in woody perennial species such as *U. minor*, for which early developmental stages are critical to survival.

As already stated, the impact of noise on seed germination appears to be related to plant life form (Phillips et al., 2021). As some relevant differences in germination are known to exist between woody and herbaceous species, it is plausible that they play a role in their response in case of environmental cues and stressful conditions (Šímová et al., 2018). Germination and emergence in woody species are typically slower than in herbaceous

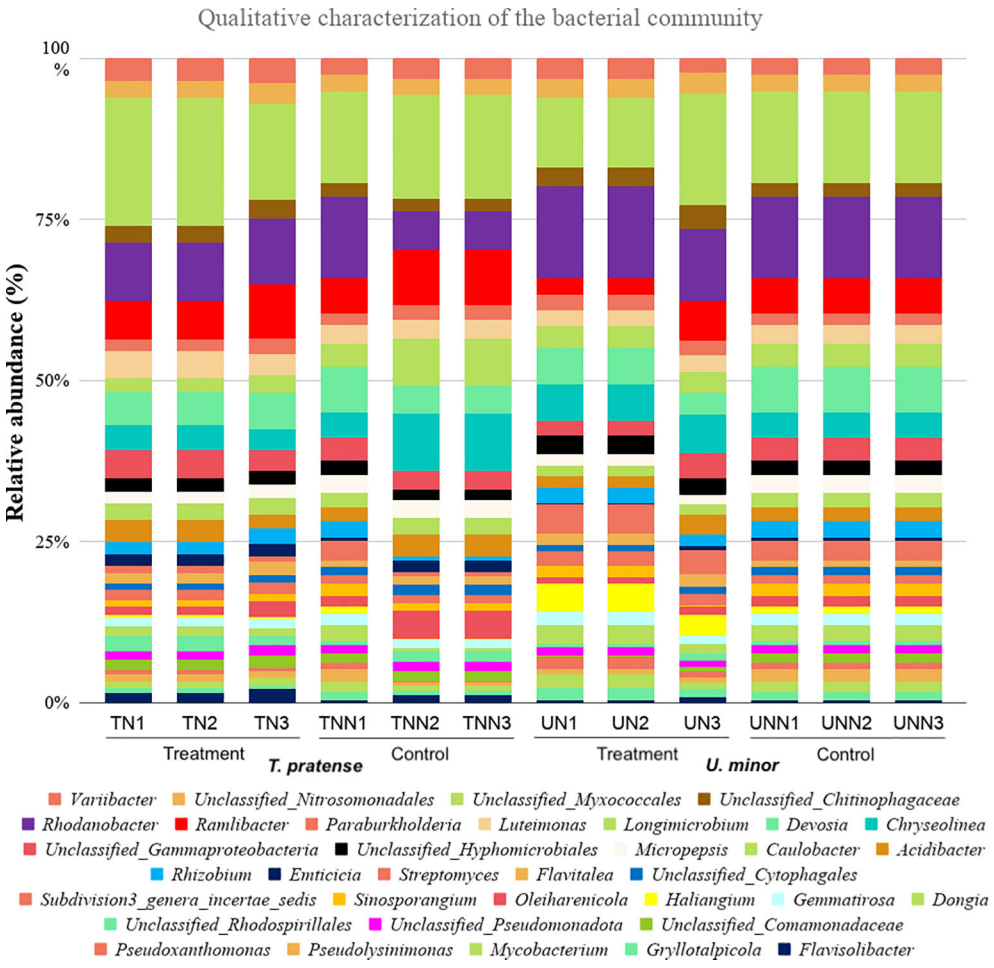

FIGURE 6
Structure (at the genus level) of the bacterial community for the two species (T, *T. pratense*; U, *U. minor*) for the six collected samples (one sample per pot, N = 6) for noise (N) and control (NN) treatments at the end of the experiment. For each genus, the relative abundance as a percentage of the total sample is indicated.

TABLE 5 Results of PERMANOVA analysis on samples collected at the end of the experiment.

Source	DF	F	P(perm)
Species (Sp)	1	2.4567	0.1246
Noise (N)	1	2.9497	0.0823
Sp x N	*2*	2.7656	0.9456
RES	5		
TOT	10		

plants and require more stable, favorable environmental conditions (Cornelissen et al., 1996). Consistent with this, no significant differences in emergence were observed for *T. pratense* between noise and control treatments, either over time or at the end of the experiment, confirming a higher tolerance to noise for this herbaceous species compared to the woody *U. minor*.

4.2 Growth and biomass allocation

Regarding growth parameters, distinct trends were observed for the two species. Specifically, data regarding the plant height, the leaf number and area, and the total dry weight were significantly lower for *U. minor* in case of noise exposure, whereas *T. pratense* showed no relevant differences for such traits in relation to the treatment. Dry weight was the only exception, showing an increase in *T. pratense* species under noise conditions.

A reduction in the main growth parameters comparable to that observed for *U. minor* has also been reported for *Salvia splendens Sellow* and *Tagetes patula L.* when exposed to traffic noise (Kafash et al., 2022). According to these authors, this trend appeared to be linked to a reduction of the concentration of plant growth-promoting hormones, which regulate cell division and expansion, and to a contextual increase in the content of stress-related hormones (Kafash et al., 2022). Moreover, beyond these changes in hormone concentrations, an increase in oxidative stress and ROS content, including H2O2 has been reported in the case of noise pollution (Li et al., 2022) for plants exposed to other stressors. Specifically, Blokhina et al. (2003) asserted that the magnitude of tolerance to oxidative damage caused by stressors is strictly related both to the species and, more generally, to the plant life-form. With this regard, Hilaire (2019) hypothesized that woody plants respond to oxidative stress more slowly than herbaceous ones, especially at their initial life stages, those considered in our study, as they are usually characterized by quick growth. Indeed, the longer life span, the more complex modularity, and the higher sectorality of woody plants compared with herbs could imply that compensatory responses and changes in the former may take several years and that the consequences of stressors such as noise are usually more relevant (Haukioja and Koricheva, 2000).

Moreover, resource allocation is a fundamental aspect of plant development and plays a key role in determining tolerance to abiotic and biotic stressors (Ruan et al., 2010). In several plants, particularly herbaceous plants, stress conditions can induce shifts in resource allocation toward increased biomass production (Cavieres and Concha-Villalobos, 2024), a response that may also occur under noise exposure. This mechanism could partly explain the significantly higher dry weight observed in *T. pratense* under noise exposure, suggesting a positive biomass response to acoustic stimulation. A similar growth enhancement has been reported in other herbaceous species exposed to specific sound frequencies. For example, Kim et al. (2021) documented an increase in different growth parameters under sound waves ranging from 250 Hz to 1500 Hz, while Cai et al. (2014) observed that mung beans (*Vigna radiata L.*) exposed to 2000 Hz exhibited enhanced seedling growth. Furthermore, sound vibrations have been found to stimulate seed germination and hypocotyl elongation in *Oryza sativa L.* (rice) and *Cucumis sativus (L.)* (cucumber) (Munasinghe et al., 2020; Johnson and Mullinix, 1998). However, prolonged exposure to high-frequency ultrasound has been shown to cause cellular damage in rice cells (Liu et al., 2003), emphasizing that the effects of sound on plants are highly context-dependent.

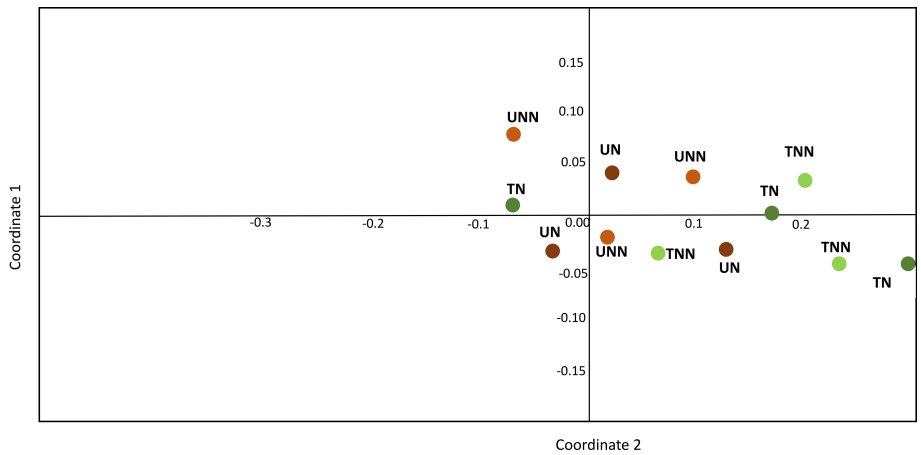

FIGURE 7
nMDS ordination of the considered samples for the two species (T: *T. pratense*; U: *U. minor*) in noise (N) and control (NN) treatments at the end of the experiment.

4.3 Photosynthetic efficiency

Plant health status, evaluated through photosynthetic efficiency, showed a significant decline in *U. minor* under noise exposure, whereas *T. pratense* maintained stable efficiency levels across treatments. Consistently, Singh et al. (2017) reported that traffic noise disrupted the physiological functioning of adult trees of *Lagerstroemia speciosa L.*, that under traffic noise reduced carbon assimilation and transpiration rate, decreased stomatal conductance, and increased stomatal resistance.

4.4 Soil bacterial community responses

Soil bacterial abundance and communities did not differ significantly between control and noise-exposed treatments, either at the beginning or at the end of the experiment. The high diversity observed (1,745 amplicon sequence variants, ASVs) suggests a complex microbial community capable of withstanding external disturbances (Delgado-Baquerizo et al., 2020). Moreover, dominant taxa, including unclassified *Myxococcales, Rhodanobacter, Ramlibacter* and *Chryseolinea* were consistently detected across treatments, supporting the idea that soil microbiomes are more resilient to acoustic stress than the plants they support. These differences can be at least partly explained by the fact that soil microbiomes are so closely associated with the soil they inhabit that they are more positively affected by the high resilience that usually characterizes it, as already stated by Griffiths et al. (2008).

Evidence on the effects of noise on soil bacterial communities remains scarce, with only a limited number of studies addressing this topic to date (Robinson et al., 2021). Available research on plants suggests that sound effects are context dependent and influenced by species- or taxon-specific traits, as well as by the amplitude, frequency, and duration of the noise exposure. For instance, For instance, one study reported an increase in the growth of *Chlorella pyrenoidosa* at optimal frequency: 0.4–1 kHz (Robinson et al., 2021), while Harris et al. (2021) observed significant biomass gains in *Saccharomyces cerevisiae* across a broad frequency range (0.1–10 kHz). Sound waves have also been shown to induce inter-species variation in microbial growth, biomass accumulation, and intracellular metabolite synthesis (Dietert and Dietert, 2024). Although positive effects, particularly under rhythmic sounds exposure, have been shown (Gu et al., 2016; Murphy et al., 2016), other studies have found no significant impact of noise on bacterial abundance or community structure (Robinson et al., 2021), in line with the results of the present study.

Our findings demonstrate that chronic exposure to broadband anthropogenic noise, simulating urban traffic conditions, acts as a species- and functional type–dependent environmental filter during early plant development. The contrasting responses of the woody *U. minor* and the herbaceous *T. pratense* highlight that noise can differentially constrain germination performance, growth, and photosynthetic efficiency without necessarily affecting short-term survival, while in some cases even stimulating biomass production. The absence of detectable shifts in soil bacterial communities further suggests that, at least over the temporal scale considered, plant responses may be more sensitive to indicators of chronic acoustic disturbance than belowground microbial assemblages. Collectively, these results underscore the potential for persistent urban noise to influence plant establishment trajectories, alter competitive dynamics between woody and herbaceous functional types, and ultimately shape vegetation structure in cities and roadside environments. Given that early developmental stages strongly determine long-term community composition and ecosystem processes, chronic acoustic pollution may represent an underrecognized driver of ecosystem functioning in increasingly noisy anthropogenic landscapes.

5 Conclusion

In conclusion, the results indicate that noise exposure elicits differential responses in plants and soil bacterial communities, depending on organism-specific traits (e.g. genotype, functional type, and physiological status) and on sound characteristics, including frequency composition, vibratory patterns, exposure duration, and environmental context. In particular, broadband, low-frequency enriched noise simulating urban traffic exerted a pronounced negative effect on woody species, whereas its impact was weaker, and in some cases neutral or positive, on herbaceous species, while no detectable effects were observed on soil bacterial communities associated with either vegetation type.

By simultaneously examining plant functional types and soil microbiomes under controlled broadband noise exposure, this study addresses a key gap in current knowledge, as most previous research has focused on single-frequency or narrow-band sounds and has rarely considered plants and soil microorganisms as an integrated system. Overall, these findings underscore the role of urban noise as a relevant environmental stressor in cities and roadside habitats, highlighting the need for species- and functional-type–specific assessments of its impacts. Future research should further disentangle noise effects by testing defined frequency bands, exposure regimes, and longer-term scenarios to improve predictions of how increasing anthropogenic noise may alter plant performance and ecosystem functioning.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: Zenodo, doi: 10.5281/zenodo.20040522.

Author contributions

VZ-C: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SCa: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SR: Data curation, Formal analysis, Investigation, Methodology, Software, Writing – review & editing. LQ: Conceptualization, Data

curation, Investigation, Methodology, Validation, Writing – review & editing. FA: Conceptualization, Data curation, Formal analysis, Investigation, Software, Writing – review & editing. EA: Data curation, Investigation, Methodology, Software, Writing – review & editing. SCi: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing. GZ: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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