

Research article

Different control strategies of the invasive plant *Arundo donax* L. have taxon-specific effects on above- and belowground biodiversity

Emanuele Fanfarillo^{a,b}, Claudia Angiolini^{a,b,*}, Leopoldo de Simone^{a,**}, Giovanni Bacaro^c, Maurizio Castaldini^d, Tiberio Fiaschi^a, Stefano Mocali^d, Emilia Pafumi^{a,b}, Francesco Vitali^d, Simona Maccherini^{a,b}

^a Department of Life Sciences, University of Siena, 53100, Siena, Italy

^b NBFC, National Biodiversity Future Center, 90133, Palermo, Italy

^c Department of Life Sciences, University of Trieste, 34127, Trieste, Italy

^d CREA - Research Center for Agriculture and Environment, 50023, Florence, Italy

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ABSTRACT

We assessed the short-term effects of different management strategies to control the invasive plant *Arundo donax* on biotic communities along a channelled stream in central Italy. We applied four treatments ordered by increasing management intensity: no management (C), one-cut per year (OC), three cuts per year (TC), and plastic mulching (M). Treatments started in June 2021 and ended in May 2022. Presence and abundance of vascular plants and soil bacteria and fungi were recorded from 24 plots immediately after the end of the treatments (May 2022) and five months later (October 2022). We tested the responses of *A. donax* and of the biotic communities to management intensity using generalized linear modeling and permutational analysis of variance. In May, M completely suppressed *A. donax* and plant communities, also reducing bacterial richness compared to C. However, plant communities under M recovered in richness by October, while *A. donax* under M and bacterial richness did not recover in such a timespan. Fungal richness was unaffected by treatments in both sampling seasons and decreased in October under all treatments. Management intensity had negative impacts on plant and bacterial functional diversity in both sampling seasons but did not affect fungal functional diversity. We highlighted that effective control of invasive species may imply short-term negative impacts on biotic communities. However, such impacts did not consistently affect different taxonomic groups. Plastic mulching should be used only in areas where rapid suppression of the invasive species is a priority, due to the short-term negative impacts on biodiversity of such practice.

1. Introduction

Invasive alien species are among the leading causes of biodiversity loss (Pyšek et al., 2020). Particularly along river systems, long-standing human pressures, such as channelization, dam construction, riparian deforestation, agriculture, and urban development have facilitated the invasion by opportunistic and non-native species (Angiolini et al., 2023; de Simone et al., 2025). These pressures may negatively impact native biological communities by altering vegetation structure and reducing the diversity and abundance of arthropods and birds (Herrera and Dudley, 2003; Mangachena and Geerts, 2017). Controlling invasive species is thus considered crucial to mitigate these impacts and restore

native biodiversity. However, there is evidence that invasive species control can also lead to unintended consequences, such as increased erosion, colonization by other non-native species, or harm to native biodiversity (Kettenring and Adams, 2011; Prior et al., 2018). Understanding both the benefits and risks of different control strategies is therefore essential to plan effective and sustainable management interventions.

The composition of soil microbial communities is influenced by various environmental factors, such as soil properties, vegetation cover, and climate (Labouyrie et al., 2024) and is indirectly impacted by plant community changes (Bardgett et al., 2013; Classen et al., 2015; Tsiafouli et al., 2015). Previous studies have demonstrated strong links between

* Corresponding author. Department of Life Sciences, University of Siena, 53100, Siena, Italy.

** Corresponding author.

E-mail addresses: claudia.angiolini@unisi.it (C. Angiolini), leopoldo.desimone@unisi.it (L. de Simone).

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aboveground plant diversity and belowground microbial diversity, particularly in the rhizosphere (Eisenhauer, 2016; Jambon et al., 2018). This suggests that vascular plant communities may serve as good indicators of soil microbial diversity (Barbato et al., 2019; Fanfarillo et al., 2023a). Thus, a change in the native component of plant communities caused by invasive plant species can alter in return the structure and function of soil microbial communities (Kourtev et al., 2002; Lazzaro et al., 2018). Although not affecting bacterial and fungal richness and evenness, it was found that invasion by alien plants increases the abundance of the most dominant fungal groups (Wang et al., 2022). Changes in composition and functionality of microbial communities in serpentine grasslands after non-native plant invasion were also observed (Batten et al., 2006). Concern has been raised about the effect of the control of alien plants on soil microbial communities, though this was highlighted to be of minor importance in the case of herbicide treatments (Weidenhamer and Callaway, 2010). However, research about the effects of mechanical and physical control of alien plants on soil microbial communities is still limited. Studies in restored tropical forests showed that mowing is less impacting than chemical control towards microbial biomass, soil respiration, and litter decomposition (Parré et al., 2023).

The giant reed (*Arundo donax* L., Poaceae) is included in the list of 100 of the World's worst invasive alien species (Lowe et al., 2000). The center of origin of this species is probably located in Central Asia between the Caspian Sea, southern Iran and the Indus Valley, from where it was introduced to the Mediterranean in the Neolithic (Hardion et al., 2014). At present days, *A. donax* is distributed globally across subtropical and warm temperate regions, with limited occurrences in equatorial and colder temperate areas (Goolsby et al., 2023). Its spread was favored by humans, which used the species for millennia in several fields, including agriculture, building industry, energy and food production, furnishing, instrument construction, medicine and veterinary (Jiménez-Ruiz et al., 2021). In Mediterranean countries such as France, Greece, Italy, Malta and Spain, it has become one of the most threatening species for the biodiversity of riparian ecosystems (Bruno et al., 2019). Due to its ability to significantly alter the structure and functions of invaded ecosystems (Richardson et al., 2000), *A. donax* is considered a "transformer species". Moreover, it is known to have allelopathic effects on other plant species (Abu-Romman and Ammari, 2015). Its rapid growth, vegetative reproduction, and ecological preference for moist sites make it widespread especially along disturbed watercourses (Jiménez-Ruiz et al., 2021). Along artificial and channeled streams, the spread of the species was promoted by humans to increase the stability of the banks through its rhizomes (Pinto et al., 2016). Conversely, it has been demonstrated that its shallow root system weakens bank stability instead of improving it (Buldrini et al., 2024). Moreover, the species provides habitat for semi-fossorial animals, which build their burrows under *A. donax* stands, further weakening the banks (Ceccato et al., 2022; Ceccato and Simonini, 2023; Coppola et al., 2025).

A range of methods is used to control *A. donax*, depending on factors such as the presence of native plants, stand size, the amount of biomass to be managed, terrain, and season (Bell, 1997). Systemic herbicides targeting the root mass are considered the most effective for controlling established *A. donax* populations (Liu et al., 2020). Other techniques include prescribed burning, mechanical removal using bulldozers, hand cutting with chainsaws, alteration of environmental conditions, mulching, and biological control using herbivorous insects (Bell, 1997; San Martín et al., 2019; Jiménez-Ruiz et al., 2021). Research assessing the effectiveness of *A. donax* control strategies typically focuses on metrics like population density (Spencer et al., 2008; San Martín et al., 2019) or its impacts on riparian vegetation, birds, and aquatic macro-invertebrates (Bruno et al., 2019). However, these studies often target specific taxa, overlooking the responses of multi-taxon biodiversity to increasing disturbance intensities by invasive species control (Bazzato et al., 2022, 2023). Notably, no study to date has tested the effects of *A. donax* control on soil microbial diversity. Given the importance of

microbiota in maintaining soil biogeochemical processes and shaping plant communities, this gap needs to be filled (Banerjee and van der Heijden, 2022; Delgado-Baquerizo et al., 2020).

In this study, we aimed to investigate the effectiveness of different management methodologies to control *A. donax* (no management, cutting once per year, cutting three times per year, and plastic mulching) and to assess their effect on the taxonomic richness and functional composition of vascular plant, soil bacterial, and soil fungal communities located in invaded sites. We aim to answer the following research questions: i) are more intensive treatments more effective in controlling *A. donax*? ii) are biotic communities affected by the treatments? iii) do biotic communities respond to the treatments in a similar way?

2. Methods

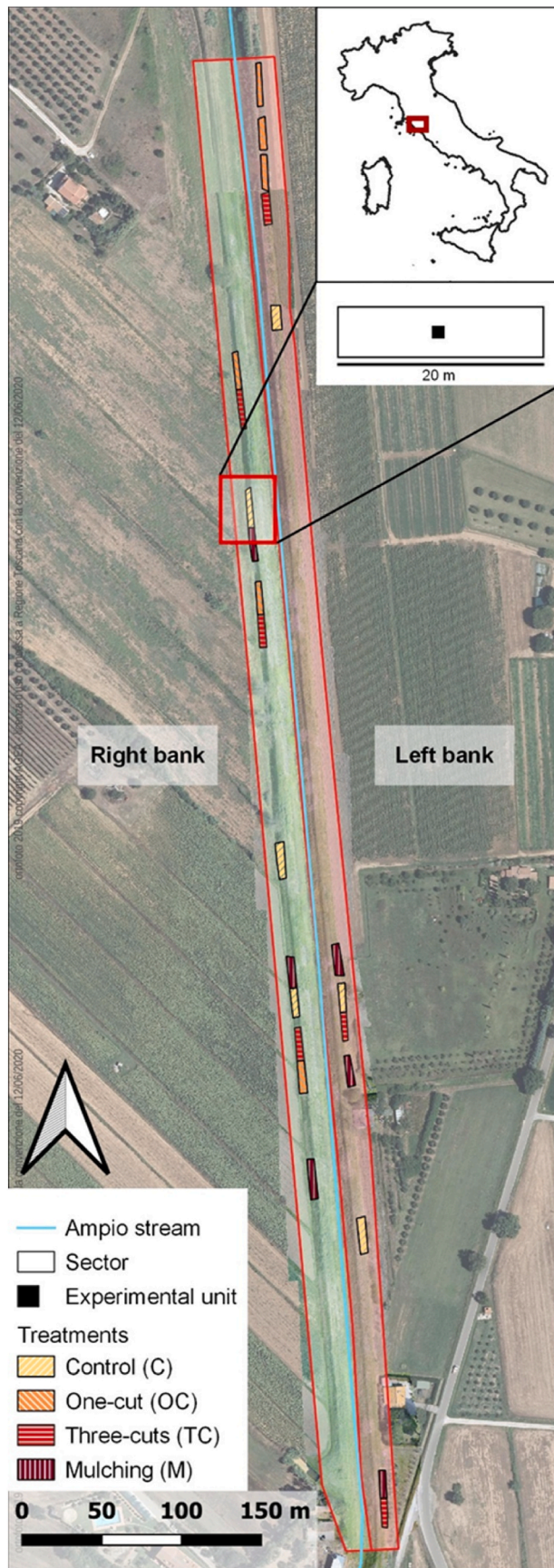
2.1. Study area and experimental design

The study was conducted along the terminal stretch of the Ampio stream (southern Tuscany, central Italy; coordinates: from 1658894.5 m E, 4740273.5 m N to 1658973.6 m E, 4739339.4 m N, EPSG 3003). The stretch is located at an elevation of 5–7 m a.s.l., within an intensively managed agricultural landscape, and it is completely channeled. Thus, both the landscape context and the stream features imply a high anthropogenic pressure in the area. The climate is Mediterranean, with a mean annual temperature of 15.7 °C and mean annual precipitations of 600 mm in Grosseto, about 10 km southeastward. Summer is the driest season, with drought conditions occurring from early June to late August (LaMMA Consortium, 2024). Soils are silty-clay-loam luvisols, with neutral or slightly alkaline pH (Hengl et al., 2017). In the area, *Arundo donax* is considered an invasive alien species (Portal to the Flora of Italy, 2024). Especially along artificial channels, *A. donax* stands provide habitat for semi-fossorial animals, whose burrowing activity combined with the plant's shallow rhizome system compromises embankment stability (Coppola et al., 2025). As a result, regular maintenance is required to manage the species. Control measures implemented by the local land reclamation consortium typically involve an annual cutting of the vegetation on the embankments.

Our study area measured 920 x 35 m. Within the study area, we set our field experiment by selecting sites dominated by *A. donax* in both the banks of the stream. *A. donax* stands, environmental conditions and vegetation management are highly homogeneous, the latter being represented by cutting once per year, and the species has been extensively present along the banks for decades. We manually delineated the *A. donax* stands from imagery acquired through an ortho-photogrammetric UAV flight, by visual interpretation in the software Agisoft Metashape (Agisoft LLC, 2023). Within the area covered by *A. donax*, a total of 24 sectors (12 per each bank of the stream), each 20 m long and with 100% *A. donax* cover, were selected. The experimental units were placed in the center of each sector, to maximize the distance between them (at least 20 m) and to minimize the possible effect of adjacent treatments. To equalize the initial conditions of the field experiment, all stands of *A. donax* within the study area were cut. Then, four treatments were applied: 1) Control (C), no management; 2) One-cut (OC), where clear-cutting occurred once during the experimental period; 3) Three-cuts (TC), involving clear-cutting three times; and 4) Mulching (M), clear-cutting once followed by mulching with a double black plastic film (Fig. 1). To take into account the possible effect of the different aspects of the two banks, which are oriented respectively toward the East and the West, the four treatments were randomized within each bank. Management started on June 18, 2021 and ended on May 13, 2022, with the removal of the mulching sheets.

2.2. Biodiversity sampling

Vascular plant, bacterial, and fungal community data were recorded in 1 × 1 m plots centered within each sector. Sampling occurred on May



Aerial view



Control (C)



One-cut (OC) / Three-cuts (TC)



Mulching (M)



Fig. 1. Study area, experimental design and treatment applications in the field.

13, 2022, after the end of the treatments, and again on October 12, 2022. For each plot, all occurring plant species were recorded, and a cover value was assigned to each on a scale from 0 to 100 %. Additionally, we collected three soil subsamples per plot for bacterial and fungal community analysis. In each plot and on both observation dates, we recorded attributes related to the condition of *A. donax*: total cover, stem height (averaged from the direct measurement of 5 culms), stem density, and stem diameter (averaged from 10 culms).

Soil samples were air dried for two days and then sieved at 2 mm. Five grams of sieved soil per sample was stored at -20°C for subsequent nucleic acid extractions via the FastDNA™ SPIN Kit for Soil (MP Bio-medicals, Santa Ana, California, USA). DNA extracts were eluted in sterile water, and their integrity was verified using 1 % w/v agarose gel electrophoresis. The DNA extracts were stored at -20°C for subsequent molecular analyses.

The microbial communities were characterized using high-throughput sequencing. For bacterial communities, the sequencing was targeted towards the V3-V4 region of the 16S ribosomal DNA gene using primers 341F (5'-CCTACGGGNGCASCAG-3') and 806R (5'-GAC-TACNVGGGTATCTAATCC-3') (Takahashi et al., 2014). Fungal communities were characterized by sequencing the ITS1 region using primers ITS1 forward (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 reverse (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). Libraries were sequenced on an Illumina MiSeq platform in 300-bp paired-end mode (IGA Technology Services s.r.l., Italy). Sequencing data were processed using QIIME2 v2019.1.0 (Bolyen et al., 2019). For Bacteria, forward amplification primers were removed from paired-end sequences, which were subsequently denoised, dereplicated, and filtered for chimeras using the DADA2 plugin (Callahan et al., 2016). Taxonomy of identified Amplicon Sequence Variants (ASVs) was assigned using the Scikit-learn with default settings (Pedregosa et al., 2011) against the SILVA v138.1. For Fungi, forward and reverse amplification primers were removed from sequences, which were subsequently clustered in Operational Taxonomic Units (OTU) using Vsearch (Rognes et al., 2016) in QIIME2 (de novo clustering) at 98 % identity. Vsearch was also used to classify the taxonomy of obtained OTUs against the UNITE database. Vascular plants were identified to species level following Pignatti et al. (2017–2019). Taxonomic nomenclature for plants followed the Portal to the Flora of Italy v. 2024.2 (Portal to the Flora of Italy, 2024).

2.3. Functional classification of plants, bacteria, and fungi

Plant, bacterial and fungal taxa were classified into functional groups to test the effects of management intensity on the functional composition of the studied communities. We opted for a functional characterization of the biotic communities since functional diversity can outperform taxonomic diversity in revealing the effects of disturbance on biodiversity (Liu et al., 2021a). Moreover, deriving ecological information from taxonomic data implies a deep knowledge of each species along with its biology and ecology (Fiaschi et al., 2023). While vascular plants are very well known in such terms, much of this information is still missing for soil microbiota (Fanfarillo et al., 2023a).

Plants were classified in functional groups commonly used to relate plant diversity to ecosystem functions like nitrogen fixation, soil stabilization, and support to pollinators (Maccherini et al., 2000; Steinauer et al., 2015): 1) annual grass; 2) perennial grass; 3) annual legume; 4) perennial legume; 5) other forb (annual); 6) other forb (perennial). Fungal and bacterial taxa were classified into functional groups using FunGuild (Nguyen et al., 2016) and Tax4fun (Aßhauer et al., 2015), respectively, allowing for an inference of metabolic/genomic functionality associated to the observed community composition. For the FunGuild results, we only considered highly probable functional attributions.

2.4. Statistical analyses

We tested the fixed effects of management intensity (four levels), sampling season (two levels - hereafter interpreted as the combined effect of the time elapsed since the end of treatments and of meteorological seasonality), bank (two levels), and their interactions on: i) *A. donax* stem density; ii) *A. donax* percent cover; iii) *A. donax* mean stem diameter; iv) *A. donax* mean height; v) plant species richness; vi) bacterial taxonomic richness (ASVs); vii) fungal taxonomic richness (OTUs); viii) functional composition of the surveyed communities.

A. donax attributes and the taxonomic richness of plant, bacterial, and fungal communities were analyzed using Generalized Linear Models (GLMs), with management intensity, sampling season, bank (left vs. right), and their two-way interactions as explanatory variables. Taxonomic richness was square-rooted for plant communities and log-transformed for bacterial and fungal communities to meet assumptions of normality. GLMs were fitted assuming Gaussian error distributions. Initial full models including all main effects and interactions were simplified through stepwise backward selection based on the Akaike Information Criterion (AIC) to derive the minimum adequate model (MAM), except in cases where full model retention was justified due to ecological or statistical considerations (e.g., when full model AIC and residual diagnostics indicated no benefit in simplification). Spatial autocorrelation of model residuals was evaluated using Moran's I statistic, based on a k-nearest-neighbour spatial weights matrix derived from geographic coordinates. The explained deviance was used as measures of model fit and explanatory power. Residual diagnostics included the Shapiro–Wilk test for normality. The coefficient of determination (R^2) and AIC values of full versus reduced models were used to assess explanatory power and parsimony. To further understand significant interactions, estimated marginal means (EMMs) were computed for selected interaction terms, and pairwise comparisons were conducted using Tukey-adjusted *p*-values. All statistical analyses were conducted in R (version 4.3.2; R Core Team, 2023). Key packages used include MASS (Venables and Ripley, 2002) for model simplification (function *stepAIC*), spdep (Pebesma and Bivand, 2023) for spatial autocorrelation analysis, performance (Lüdtke et al., 2021) and rsq (Zhang, 2024) for model diagnostics and R^2 calculations, DHARMA (Hartig, 2024) for additional residual simulations when necessary, and emmeans (Lenth, 2024) for post-hoc pairwise comparisons.

As regards functional composition, we tested the effects of the predictor variables using permutational analysis of variance (PERMANOVA - Anderson, 2001) in the software PRIMER v.7 (Clarke et al., 2014). Dissimilarity matrices were built using Bray-Curtis distance, adding a dummy species when empty plots were present in the plant matrix. Pairwise comparisons were conducted using *t*-tests. Differences in functional composition were then visualized with Non-metric Multidimensional Scaling (NMDS) ordinations using the function *metaMDS* in the vegan package of R (Oksanen et al., 2020). The tests were carried out with 999 permutations of residuals under a reduced model (Anderson and Ter Braak, 2003), setting the significance level (α) at 0.05.

For all the analyses, if the factor management intensity, sampling season or the interaction management intensity \times sampling season were significant, we carried out pairwise post-hoc tests between seasons within levels of management intensity and between levels of management intensity within seasons (Wei et al., 2012).

3. Results

3.1. *A. donax*

Sampling season had a significant effect on *Arundo donax* density, diameter, and cover, while management intensity significantly affected all the dependent variables. Bank was significant for density, diameter, and height. The interaction between sampling season and management intensity was significant only for density and height. The interaction

between management intensity and bank significantly affected density, diameter, and height. No spatial autocorrelation of residuals was detected for any model and all residuals were normally distributed (Table 1).

From May to October, density decreased under OC, cover increased under TC, and diameter decreased under C. In both seasons, all the attributes were reduced to zero under M. Density significantly decreased under M in both seasons. Cover decreased under TC compared to C and OC and showed a further significantly lower value under M in May, while it was significantly lower only under M in October. In both seasons, diameter significantly decreased from C to OC and TC, further decreasing under M. In May, height significantly decreases from C to OC and from OC to TC and M, while it decreases from C to TC and from TC to M in October (Fig. 2).

3.2. Taxonomic richness

Sampling season had a significant effect on the richness of all the three taxonomic groups, while management intensity only affected plants and bacteria. Bank significantly affected only plant species richness. The interaction between sampling season and management intensity significantly affected plant species richness. No spatial autocorrelation of residuals was detected for any model and all residuals were normally distributed (Table 2).

From May to October, plant species richness significantly increased under M. Fungal OTU richness significantly decreased in October under all treatments. In May, plant species richness significantly increased from C to TC, and it became zero under M. In October, plant species richness increased from C to TC and M. Bacterial ASV richness was lower under M compared to C in both seasons (Fig. 3).

3.3. Functional composition

Management intensity and sampling season significantly affected the functional composition of plant and bacterial communities, but not that of fungal communities. Moreover, the interaction between management intensity and sampling season significantly affected plant community functional composition. Bank was not significant for any of the treatments (Table 3).

Plant community functional composition differed between May and October under OC, TC, and M. Namely, annual species were more abundant in May and perennial species were more abundant in October. In May, plant community functional composition significantly differed between M and the rest of the treatments. The same differences were highlighted in October. In May, bacterial community functional composition significantly differed between C and OC and between C and TC. In October, it only differed between C and M.

Fig. 4 shows the NMDS ordinations highlighting the functional changes of plant and bacterial communities according to management

intensity and sampling season. In both groups, functional composition changes were mostly linked to a decline in the abundance of taxa with specific functions in the M plots, since there were no functions related to the latter. This pattern was observed both in May and in October.

4. Discussion

We had evidence that more intensive treatments are more effective in controlling *A. donax* and affect only plant and bacterial communities. Confirming previous evidence, our study showed how mulching was highly effective in suppressing *A. donax* in the short term. However, it also revealed mainly negative effects of this practice on plant and bacterial communities, with a decrease in taxonomic richness and a loss in functionality. We also highlighted that plants, bacteria and fungi are differently affected by the treatments both in terms of taxonomic richness and functional composition. Moreover, the investigated communities showed different resilience after the ceasing of management practices, with plants showing the highest resilience. Conversely, fungal communities were the most resistant, not showing any change in both taxonomic richness and functional composition across all the duration of the experiment. We also showed how the influence of the sampling season and of the time elapsed since the end of treatments should be considered when monitoring the effects of containment practices on both *A. donax* and biotic communities over time.

4.1. Effectiveness of treatments in containing *A. donax*

After the cessation of treatments, *A. donax* did not recover in any of the measured attributes after five months in the plots under mulching. Similarly, the effect of the other treatments, such as the decrease of cover, diameter, and height, persisted at the end of the observation period. These results are partially consistent with previous evidence. In a field experiment in the USA, 1-year observations under cutting and mulching treatments revealed that mulching was the only successful technique to control the species, among mechanical and physical ones (San Martín et al., 2019). On the contrary, cutting could even favor the spread of the species if residues, and especially rhizomes, are not removed from the ground, due to their resprouting abilities (Calazans et al., 2023). An increase of cover under TC was also observed over the growing season, since ground cutting stimulates a more vigorous regrowth of the plant (Racelis et al., 2012). Cutting alone is considered the least effective control strategy, while integrated management is recommended, including biological control through the wasp *Tetramesa romana* and chemical control through glyphosate (Jiménez-Ruiz et al., 2021). Based on our results, we recommend mulching as a priority management option in sites where physical treatments are feasible and where a rapid reduction of *A. donax* biomass is necessary. However, this practice can be logistically and economically demanding, particularly in large-scale invasions where full extensive coverage may be impractical.

Table 1

GLM models on the effects of management intensity, sampling season, bank, and their two-way interactions on *Arundo donax* density, diameter, height, and cover; df = degrees of freedom; MAM = Minimum Adequate Model; AIC = Akaike Information Criterion.

	df	Density (Full model)	Cover (MAM)	Diameter (MAM)	Height (Full model)
Sampling season	1	1552.70***	2002**	0.85**	8112, n.s.
Management intensity	3	8587.70***	45,721***	26.77***	900,055***
Bank	1	1887.50***	19, n.s.	0.55**	14,255*
Sampling season × Management intensity	3	899.10*	1227, n.s.	0.37, n.s.	37,366*
Sampl season × Bank	1	553.50*	533, n.s.	–	5870, n.s.
Management intensity × Bank	3	1001.20*	–	0.85*	66,374**
R ²	–	0.83	0.86	0.93	0.90
AIC (MAM/Full model)	–	–	404.61/407.82	17.90/19.90	–
Moran's I	–	–0.03, n.s.	–0.15, n.s.	–0.06, n.s.	–0.22, n.s.
Shapiro-Wilk	–	0.96, n.s.	0.98, n.s.	0.97, n.s.	0.96, n.s.

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

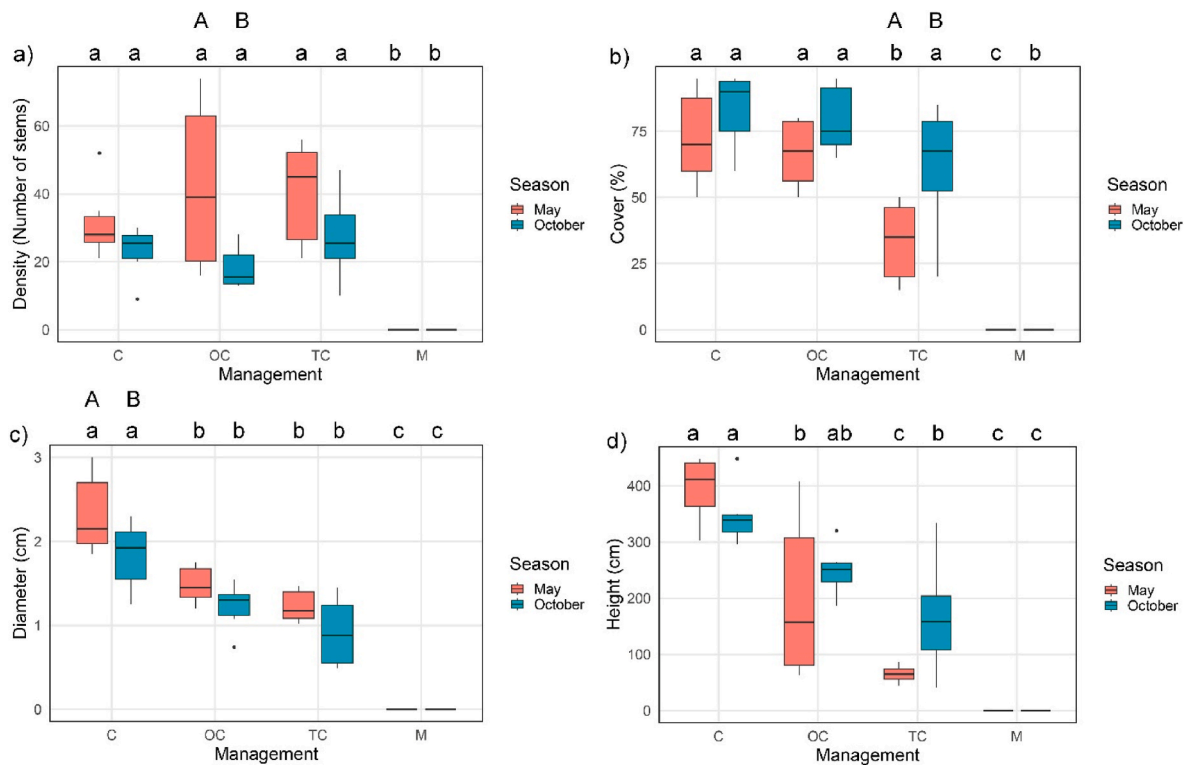


Fig. 2. Boxplots showing differences in *A. donax* a) density, b) cover, c) diameter, and d) height between sampling seasons and treatments. Different capital letters indicate statistically significant differences between seasons, within treatments, and different lower-case letters indicate statistically significant differences between treatments, within seasons (Tukey test).

Table 2

GLM models on the effects of management intensity, season, bank, and their two-way interactions on plant species richness, bacterial ASV richness, and fungal OTU richness; df = degrees of freedom; MAM = Minimum Adequate Model; AIC = Akaike Information Criterion.

	df	Plants (MAM)	Bacteria (MAM)	Fungi (MAM)
Sampling season	1	2.95***	0.66*	12.37***
Management intensity	3	17.65***	1.36**	–
Bank	1	0.64*	0.23, n.s.	–
Sampling season × Management intensity	3	29.27***	–	–
Sampling season × Bank	1	–	0.18, n.s.	–
R ²	–	0.92	0.37	0.73
AIC (MAM/Full model)	–	42.52/ 48.99	33.93/40.51	29.23/ 42.91
Moran's I	–	0.12, n.s.	–0.13, n.s.	–0.17, n.s.
Shapiro-Wilk	–	0.98, n.s.	0.98, n.s.	0.98, n.s.

* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

4.2. Impacts of management intensity on taxonomic richness

In May, plant species richness showed an increasing trend from C to TC. Conversely, the M treatment led to the disappearance of all vascular plant species. This pattern can be explained by the reduced competition of *A. donax* on other plant species with increasing cutting frequency, but not under M, which killed any present plant. An increase in plant species richness with increasing cutting frequency of *A. donax* was already highlighted in a Spanish wetland ecosystem (Bruno et al., 2019). From this perspective, the frequent cut of *A. donax* can increase the levels of plant diversity and could be considered more sustainable than plastic mulching in the short term. In October, plant species richness showed a positive response to increasing management intensity, indicating that plant communities had a high resilience, which was particularly evident

under M. This could be explained by pioneer, ruderal annual plant species like *Amaranthus retroflexus*, *Avena sterilis*, and *Sonchus oleraceus* promptly colonizing the bare ground after mulching removal (Prach et al., 2001).

Bacterial communities showed a decrease in taxonomic richness under the M treatment in both seasons. A decrease in bacterial richness under plastic mulching was previously highlighted in *Phyllostachys praecox* plantations and wheat fields in China (Gao et al., 2024; Zhang Y. et al., 2024). In the first case, this effect was linked to a reduction in soil oxygen content. In fact, most bacteria prefer O₂-rich substrates (Baez and Shiloach, 2014). Conversely, our results are in contrast with previous findings that both biodegradable and non-biodegradable plastic mulching increase taxonomic richness in soil bacterial communities (Xue et al., 2022). The negative effect of M on bacterial richness persisted in October, suggesting a low resilience of such communities in the short term, differently from what we observed for plants.

Fungal taxonomic richness was not affected by any treatment, either in May or October. Previous research assessing the effects of *A. donax* cutting on soil fungal richness is not available. As regards mulching, studies mostly focused on the effect of plastic mulching residues in soils and usually highlighted no effects or a reduction in fungal richness (Santini et al., 2024; Song et al., 2024). The high resistance of fungal communities in terms of taxonomic richness may be since our treatments were applied on a reduced surface, while the extent of fungal underground networks is much wider, connecting multiple plants to take nutrients from and possibly promoting such resistance (Liu et al., 2021b). The decrease in fungal richness under all treatments across the season is likely due to natural seasonal fluctuations, as highlighted in other temperate ecosystems (Zhao et al., 2023).

Microbial communities showed different responses to management intensity. Within soil microbiota, bacteria are more sensitive to changed environmental conditions compared to fungi. This is due to their higher turnover rates and quicker reaction to physical and chemical shifts in the soil ecosystem (Zhou et al., 2013; Glassman et al., 2018). Previous

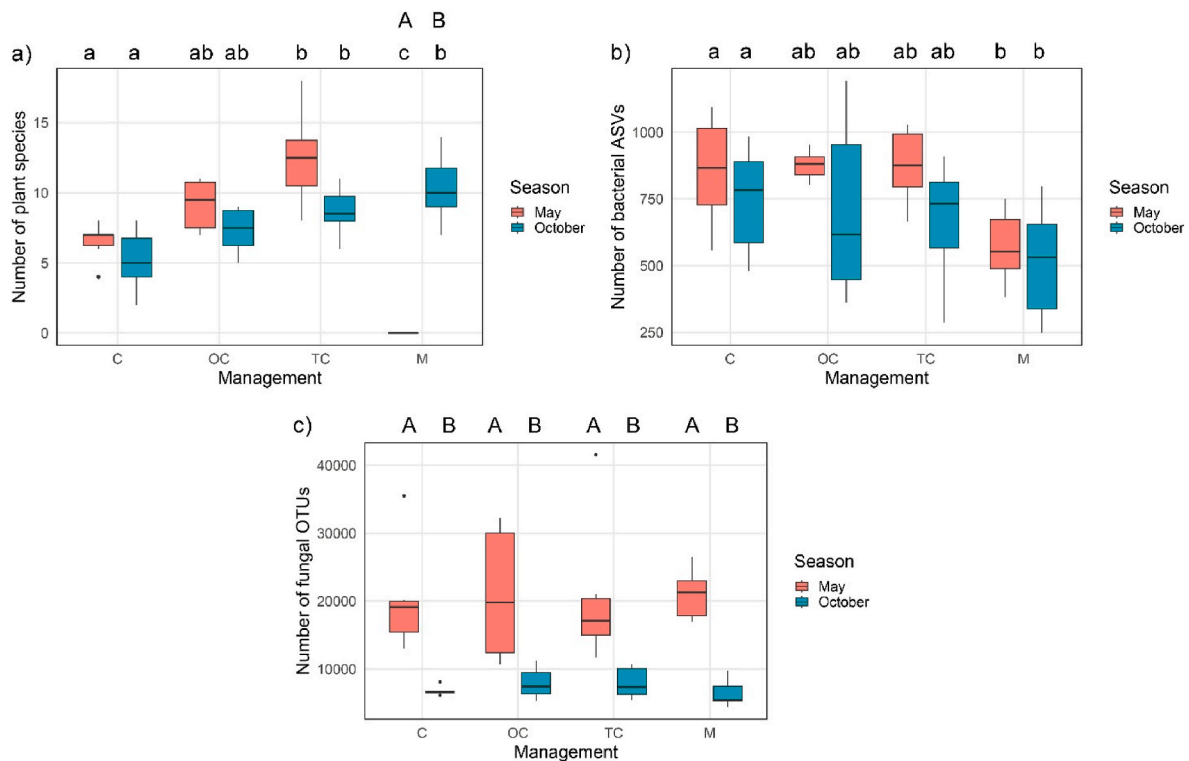


Fig. 3. Boxplots showing differences in a) plant, b) bacterial, and c) fungal taxonomic richness between seasons and treatments. Different capital letters indicate statistically significant differences between seasons and different lower-case letters indicate statistically significant differences between treatments (Tukey test).

Table 3

PERMANOVA results showing the effects of management intensity, sampling season, bank, and their interactions on the functional composition of plant, bacterial, and fungal communities.

Source of variation	df	Plants			Bacteria			Fungi		
		SS	MS	Pseudo-F	SS	MS	Pseudo-F	SS	MS	Pseudo-F
Sampling season	1	16,384	16,384	10.05**	1479	1479	4.01*	541	541	0.68
Management intensity	3	13,363	4454	2.73**	3015	1005	2.72*	3070	1023	1.29
Bank	1	2678	2679	1.64	317	317	0.86	1429	1429	1.8
Sampling season x Management intensity	3	30,116	10,039	6.16**	663	221	0.6	2808	936	1.18
Sampling season x Bank	1	995	995	0.61	323	323	0.88	481	481	0.61
Management intensity x Bank	3	3977	1326	0.81	760	253	0.69	2048	683	0.86
Sampling season x Management intensity x Bank	3	2592	864	0.53	1371	457	1.24	1353	451	0.57
Residuals	32	52,189	1631		11,810	369		25,415	794	
Total	47	122,290			19,738			37,144		

** = $p < 0.01$; * = $p < 0.05$.

studies have shown that human impacts can affect bacterial and fungal richness in different ways, but with contrasting and context-dependent results. For example, human density increased bacterial but not fungal richness in forest ecosystems (Chen et al., 2020), while land-use intensity decreased primary producers but increased fungal pathogens, fungal decomposers, and bacterial decomposers in agricultural grasslands (Le Provost et al., 2021). In other works, plastic mulching decreased fungal richness but increased bacterial Shannon diversity (Liu et al., 2024). This higher resistance of soil fungi may be since they can use soil organic carbon more efficiently than bacteria (Grover et al., 2015). Despite this, there was also evidence that plastic film mulching decreases fungal richness by increasing soil moisture and promoting soil organic matter decomposition (Liu et al., 2021b). In this study, low levels of disturbance resulted in minimal or no response from the analyzed taxa, consistently with findings by Maccherini et al. (2021) in a forest ecosystem subjected to thinning. However, the drastic M treatment, which prevents light penetration and increases soil temperature, significantly decreased bacterial richness in May.

4.3. Impacts of management intensity on functional composition

The impact of management intensity on the functional composition of the biotic communities was similar to the one observed for taxonomic richness. Plant community functional composition changed across the sampling season. Management intensity significantly altered plant and bacterial functional composition, with mulching being the most impactful treatment. Conversely, fungal communities remained unaffected, highlighting differing resistance between the three groups.

The shift towards a higher abundance of perennial plant species in October can be related to the increased temporal distance from the end of the treatments and disturbance. This pattern is well-known from previous studies in semi-natural grasslands (Maccherini and Santi, 2012; Fanfarillo et al., 2023b). In May, the shifts caused by increasing intensity on plant community functional composition were basically due to the disappearance of all plant species, and then of any functional group, under the M treatment. Black plastic mulching is highly effective in suppressing herbaceous plant species, killing all of them during the

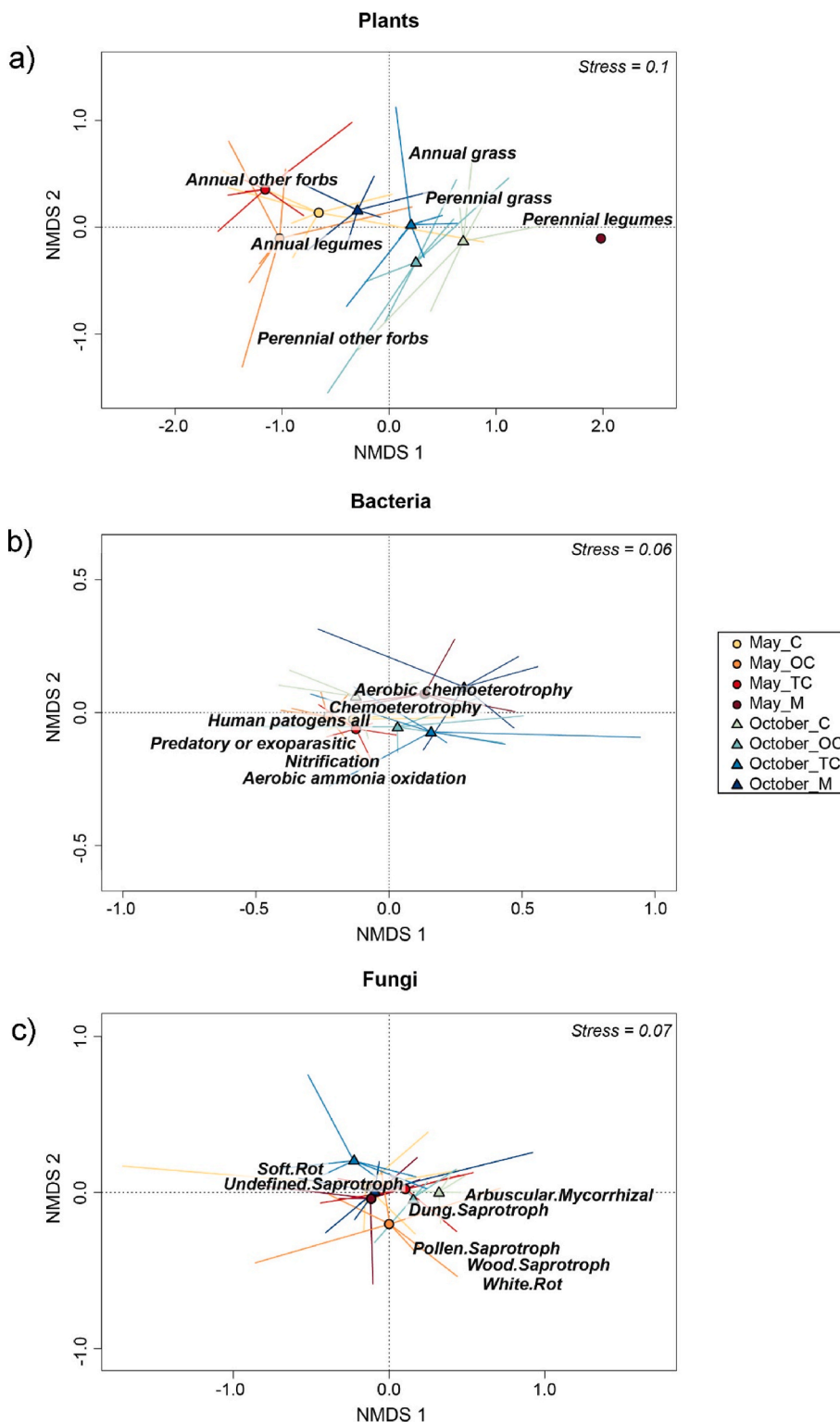


Fig. 4. NMDS ordination showing the changes in functional composition of a) plant, b) bacterial, and c) fungal communities according to management intensity and sampling season; C = control; OC = one cut; TC = three cuts; M = mulching; spiderplots highlight plots under the same management intensity in each season (May or October; circles and triangles = group centroids; lines link each plot with the group centroid).

application (Jabran, 2019). The differences in plant functional composition observed in October between M and the rest of the treatments were instead due to the colonization of bare soil in M plots by pioneer annual species, while plots under the other treatments had a higher presence of perennials in response to the lower disturbance (Symonides, 1988).

Our results showed that all the functional groups of bacteria were negatively affected by M. Such functional shifts can be attributed to the sensitivity of soil bacteria to micro-environmental changes such as altered organic matter, moisture, and aeration caused by mulching (Fierer and Jackson, 2006). Previous evidence shows that plastic mulching can functionally alter soil bacterial communities in several

ways. For instance, plastic mulching has been associated with an increase of denitrifying bacteria in a field experiment in China (Dong et al., 2024). Other evidence showed how plastic mulching increased the abundance of bacterial functional genes that are involved in ammonification (Dou et al., 2023). Microplastics derived from mulching sheets could also induce functional changes in the bacterial community, since they act as a distinct microhabitat for soil bacteria (Zhang et al., 2019).

Differently from plants and bacteria, fungi demonstrated remarkable resistance, maintaining stable functional patterns throughout the experiment. Similarly to what was observed for fungal richness, this stability could be driven by their extensive mycelial networks (Liu et al., 2021b) and functional redundancy (Gosling et al., 2016), which make fungi less susceptible to the locally applied treatments. Our results are consistent with previous evidence that highlighted a resistance of soil fungal communities to short-term warming, like that induced by plastic mulching, though variations in functionally important taxa such as those involved in CO₂ efflux rates were observed (Xiong et al., 2014).

5. Conclusions and management implications

In this study, we show for the first time that mechanically or physically managing the invasive species *Arundo donax* has varying impacts on biodiversity, with taxon-specific responses. Increasing management intensity had similar negative effects on some groups, including reductions in plant and bacterial richness and shifts in their functional composition. However, plant communities showed signs of recovery five months after the cessation of treatments. Conversely, fungal communities were unaffected by treatments, showing high resistance both in terms of taxonomic richness and functional composition. Based on this evidence, we recommend that strategies for controlling invasive plant species should be designed with consideration of their potential impacts on biodiversity. Control plans should include assessments of vascular plants and bacteria, given the negative impacts highlighted by our results. Since the experiment was conducted in a single study area, repeating the study in different climatic and geographic contexts and including more taxonomic groups would help generalize the observed patterns.

Our results suggest that while mulching is an effective non-chemical strategy for suppressing *A. donax*, its application should be carefully planned in space and time to account for its negative impact on plant and bacterial diversity. Mulching should be considered primarily in sites where rapid control of the invasive species is needed, given the short-term biodiversity loss that it causes.

CRediT authorship contribution statement

Emanuele Fanfarillo: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Claudia Angiolini:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Leopoldo de Simone:** Writing – review & editing, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Giovanni Bacaro:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Maurizio Castaldini:** Writing – review & editing, Validation, Data curation. **Tiberio Fiaschi:** Writing – review & editing, Investigation, Data curation. **Stefano Mocali:** Writing – review & editing, Data curation. **Emilia Pafumi:** Writing – review & editing, Software, Formal analysis, Data curation. **Francesco Vitali:** Writing – review & editing, Writing – original draft, Validation, Software, Formal analysis, Data curation. **Simona Maccherini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Claudia Angiolini, Leopoldo de Simone reports financial support was provided by Consorzio di Bonifica 6. Emanuele Fanfarillo, Claudia Angiolini, Emilia Pafumi, Simona Maccherini reports financial support was provided by European Union. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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