

RESEARCH ARTICLE

Nitrogen-fixing cushion *Astragalus siculus* modulates soil fertility, microclimate, plant facilitation, bacterial and fungal microbiota along an elevation gradient

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Abstract

Question: Cushion-forming vascular plants are adapted to alpine environments and act as ecosystem engineers by moderating microclimate and creating a fertility island under their canopy. Most of the available studies on cushion ecology have been devoted to plant facilitation, but none used a holistic approach that considers the response of co-existing plant species with other key ecosystem players. Here, we quantified the biogenic effect and facilitation capacity of *Astragalus siculus*, a spiny nitrogen-fixing cushion plant, and test the hypothesis that the facilitation effect would increase with elevation as stress gradient.

Location: Mount Etna, the highest active volcano in the Mediterranean Basin.

Methods: We combined chemical characterization of the soil with a description of the soil microbiota by high-throughput sequencing of bacterial and eukaryotic rRNA gene markers carried out at three elevations, that is 1650, 1900 and 2210 m a.s.l. In addition, we analysed the microclimate outside and inside the *Astragalus* canopy to assess the linkages between above- and below-ground ecosystem compartments and quantified the occurrence of co-existing vascular plants.

Results: *Astragalus* cushions have strong biogenic effects on above- and below-ground conditions by dramatically increasing soil organic carbon and total nitrogen stocks at 1900 and 2210 m elevation. Moreover, a buffering of soil temperature and moisture regimes was recorded, with lower temperatures in summer but higher in winter inside the cushions. Cushions harbour a specific bacterial and fungal microbiota compared to external bare soil. Stem density and cushion height increase significantly with altitude, as does the nurse effect of the cushion on the growth and survival of co-existing vascular plants.

Conclusions: As a result of cushion biogenic environmental changes, the effect of *Astragalus* on some of the co-existing species shifted from negative or null at 1650 m to facilitative along the elevation gradient. Furthermore, our data show a relationship

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between *Astragalus* morphological traits and soil biogenic changes related to soil chemistry and microbiota that affect plant interaction and coexistence.

KEYWORDS

biodiversity conservation, endemic species, Etna, fertility island, soil fungi and bacteria, soil temperature and moisture, vascular plants

1 | INTRODUCTION

As sessile organisms, plants modify the surrounding biotic and abiotic environment during their lifespan by altering organic carbon and nutrient cycling (Barot et al., 2007), regulating microclimatic conditions under their canopy (Rita et al., 2021), and selecting the associated microbiota in the phyllosphere and rhizosphere (Dini-Andreote & Raaijmakers, 2018). When plants are sufficiently large or long-lived and cause significant change in the surrounding environment, they may be referred to as 'foundation species' or 'ecosystem engineers' (Jones et al., 1997). Ecosystem engineers are defined as organisms that indirectly or directly modulate the bioavailability of resources to other species, thereby causing changes in the physical state of biotic and/or abiotic materials (Jones et al., 1994). Among ecosystem engineers, plants can exert their effects on biological communities through a variety of mechanisms. For example, plants can generate positive feedbacks for their own benefit by buffering temperature extremes and winds or increasing soil moisture and fertility. Moreover, by buffering stressful conditions, ecosystem engineers can also create suitable conditions that facilitate the establishment and growth of other plant species; in this case, these species are also referred to as nurse plants (Callaway, 2007).

Cushion-forming species, hereafter referred to as cushions, exhibit a growth form typically due to plant adaptation to windswept sites and extreme habitat conditions. The growth form of cushions is common in mountaintop and circumpolar environments and acts as a special kind of ecosystem engineer: its domed shape moderates the temperature ranges inside the canopy and mitigates moisture fluctuations (Körner, 2021). Moreover, cushion-forming species can occur on exposed coasts, shallow soils, edges of salt lakes and sand plains in semi-deserts and seasonally dry ecosystems (Quezel, 1967; Zohary, 1973; Guarino et al., 2006). Cushions can act as air filters, retaining dust and litter and potentially any type of wind-dispersed particles, resulting in a significant local improvement in soil fertility (Aubert et al., 1914). Thanks to these properties, cushion plants can serve as nurse-like microenvironments not only for vascular plants but also for bacteria, fungi, and arthropods (Molenda et al., 2012). Most of the available studies on cushion ecology have been devoted to plant facilitation (Reid et al., 2010), with several studies investigating causal mechanisms, including the buffering effect on microclimate and the formation of a fertility island (Zhao & An, 2021).

Much less attention has been paid to the potential role of the soil microbiota in mediating the cushion-induced facilitation effect on other plants (Rodríguez-Echeverría et al., 2016). An early study

reported that *Silene acaulis* (L.) Jacq. cushions harbour a specific fungal microbiota, but the potential implications for plant-plant interactions were not investigated (Roy et al., 2018). Recently, Liu et al. (2023) reported that the alpine cushion *Thylacospermum caespitosum* (Cambess.) Schischk. in northwestern China has a specific soil microbiota in terms of bacterial and fungal composition that has cascading effects on nurse capability. In general, plants are known to alter soil chemical profiles and shape associated bacterial and fungal communities, with species-specific microbiota signatures found for shrublands (Idbella et al., 2022), and forests (Baldrian, 2017). Concerning alpine cushions, Wang et al. (2020) reported that the different phenotypes of *Thylacospermum caespitosum*, that is, loose, tight, as well as microtopographic sites affect the bacterial and fungal microbiota. However, despite the growing literature on soil microbiota associated with nurse plants (Rodríguez-Echeverría et al., 2016), no study has examined whether bacterial and fungal communities change along elevation gradients. To check how microbial communities may shift in response to changing environmental conditions could provide valuable insights into ecosystem functioning and biotic interactions in mountain environments.

The stress-gradient hypothesis (SGH) developed by Bertness and Callaway (1994) states that the intensity and type of interaction between plant species changes along a gradient of environmental stress. Positive interactions are more common as environmental stress increases and the facilitation by nurse plants increases with elevation. Mechanistically, this occurs because positive plant-plant interactions gradually outweigh the competition for resources, which is more intense under favourable conditions (Brooker & Callaghan, 1998). However, several results support an alternative view to the SGH model of interaction, in which plant facilitation breaks down at the upper end of the abiotic stress gradient because nurse traits are unable to provide suitable conditions for plant establishment and growth (Michalet et al., 2006; Holmgren & Scheffe 2010). In fact, the ability of a plant to modify its environment and thus act as a putative nurse plant is influenced by its structure and morphology (Schöb et al., 2013). In general, plant morphology can change along environmental gradients as a result of differences in intraspecific genetic traits or as a result of phenotypic plasticity (Henn et al., 2018). In addition, the biological ability of cushion plants to act as nurses, by promoting positive interactions with other plants species, may change along environmental gradients as a function of morphological parameters (Anthelme et al., 2017). For example, the morphology of *Silene acaulis* changes greatly along an elevation gradient of 700m, evolving from a lax, soft, and flat-shaped cushion



at low elevations to a tightly knit and dome-shaped habit at high elevations (Bonanomi et al., 2016). However, very few data are available on the changes in soil microbial communities and soil properties along an elevation gradient (Ren et al., 2018). Overall, it seems clear that further work is needed to establish a mechanistic link between plant morphological traits, associated biogenic soil properties, microclimate buffering, and variations in net plant-plant interactions along environmental gradients.

Previous studies have focused on the facilitative effects of cushion plants on native or exotic species (Arredondo-Núñez et al., 2009), with in-depth studies examining their effects on soil and above-ground microclimate (Körner, 2021), changes in soil chemistry (Zhao & An, 2021), or associated microbiota using the next-generation sequencing approach (Roy et al., 2018). Nevertheless, none of the previous studies on cushion plants adopted a holistic approach that simultaneously examined the effects of an ecosystem engineer on soil properties, bacterial and fungal microbiome, microclimate, and their combined effects on co-existing species. Here, our general objective is to evaluate the overall biogenic effect and facilitation capacity of *Astragalus siculus* Biv. (hereinafter *Astragalus*), a spiny nitrogen-fixing cushion plant endemic to Mt Etna, in southern Italy. Our specific hypothesis was that the facilitation effect of *Astragalus* would increase with elevation according to the SGH model. To obtain a complete view of the causes of *Astragalus* facilitation, we combined chemical characterization of the soil, that is, particle size distribution, organic carbon, and total nitrogen, with a description of the soil microbiota by high-throughput sequencing of bacterial and eukaryotic rRNA gene markers. In addition, we analysed the microclimate under the *Astragalus* canopy to assess the linkages between above- and below-ground ecosystem compartments. The specific objectives of our study were to: (i) quantify the effects of *Astragalus* canopy on microclimate and soil chemistry; (ii) investigate the relationships between changes in abiotic factors caused by *Astragalus* and soil bacterial and fungal microbiota; and, (iii) evaluate the facilitation of vascular plants by *Astragalus* along an elevation gradient.

2 | MATERIALS AND METHODS

2.1 | Study site description

This study was conducted on Mt Etna, the highest (3357 m a.s.l.) active volcano in the Mediterranean Basin (eastern Sicily; 37°45.3' N, 14°59.7' E). The Etnan landscape is characterized by lava flows of different ages (Branca, Coltelli, GropPELLI, & Lentini, 2011), corresponding to a wide array of different land covers and traditional land uses (Poli-Marchese & Patti, 2000; Barbera et al., 2004).

The elevation of our study sites ranged between 1650 m and 2210 m a.s.l. on the northeastern slope of the mountain. The soil has andic properties and can be classified as Vitric Andisols (Soil Survey Staff, 2010) laying over thick pyroclastic flow and fall deposits interbedded in the lava succession of Serra delle Concazze and Pizzi Deneri Formations (Branca, Coltelli, & GropPELLI, 2011). According

to the bioclimatic classification by Rivas-Martínez et al. (2002), the study site falls within the upper supra- and oromediterranean humid bioclimatic belts (Bazan et al., 2015). At the nearest weather station (Piano Provenzana, 1825 m a.s.l.; 2.3 km northwards from our study site), the mean annual temperature is 8.4°C and mean annual rainfall amounts to 930 mm, with a pronounced summer drought.

The plant community targeted in our study was described as *Astragaletum siculi* (Frei 1940) Gilli 1943 and forms a patchy, discontinuous layer dominated by *Astragalus siculus*, with scattered presence of (among the others): *Bellardiachloa variegata* (Lam.) Kerguelen subsp. *aetnensis* (C. Presl) Giardina & Raimondo, *Cuscuta epithimum* L. subsp. *kotschyi* (Des Moul.) Arcang., *Erysimum etnense* Jord., *Galium aetnicum* Bivona, *Robertia taraxacoides* (Loisel.) DC., *Rumex scutatus* L. subsp. *aetnensis* (C. Presl) Cif. & Giacom., *Senecio squalidus* L. subsp. *aethnensis* (Jan ex DC.) Greuter, *Tanacetum vulgare* L. subsp. *siculum* (Guss.) Raimondo & Spadaro. This vegetation thrives on pyroclastic deposits and stony slopes in windy sites between 1500 and 2200 m a.s.l., but it can be also found at lower elevations, up to 1000 m, in disturbed sites (Brullo et al., 2005).

2.2 | *Astragalus siculus* plant profile

Astragalus siculus (\equiv *Astracantha sicula* (Biv.) Greuter ex Reer & Podlech) (Fabaceae) is a dwarf cushion-forming subshrub (pulvinate chamaephyte), up to 60 cm tall, densely branched from the base, with stems prostrate or ascending, each ending with a terminal rosette formed by paripinnate leaves with persistent thorny rachis (Figure 1). The flowers are papilionaceous with a pink corolla and appear in summer, from June to mid-July (Pignatti et al., 2019). This species is exclusively found on Mt Etna (Sciandrello et al., 2020) and it is part of the regional endemic elements of the Italian flora (Stinca et al., 2021). Based on the IUCN criteria, it was assessed as Near Threatened (Orsenigo et al., 2018) by anthropic activities (e.g., tourism and fire) and natural disturbance related to the volcanic fallouts.

2.3 | Vegetation survey and *Astragalus* traits

In mid-July 2018, we randomly selected 15 *Astragalus* cushions at three different elevations (1650, 1900, and 2210 m a.s.l.), for a total of 45 cushions. Moreover, an additional 45 adjacent open areas were randomly selected, with a minimum distance of 2 m and a maximum distance of 5 m from the cushions. Species accumulation curves ensured that the sampling effort was sufficient to represent the plant community (Appendix S1). At each site, we quantified the relative abundance of all vascular plant species within paired adjacent plots of 200 cm \times 200 cm. We selected this size for outside plots because it is comparable to the size of the *Astragalus* cushions, which average 182 cm in diameter. Plant species cover was visually estimated, based on the following abundance scores: 1 (rare), 2 (<1%), 3 (1%–5%), 4 (6%–15%), 5 (16%–25%), 6 (26%–50%), 7 (51%–75%), 8 (76%–100%). The taxonomic identification of the observed plants was based on

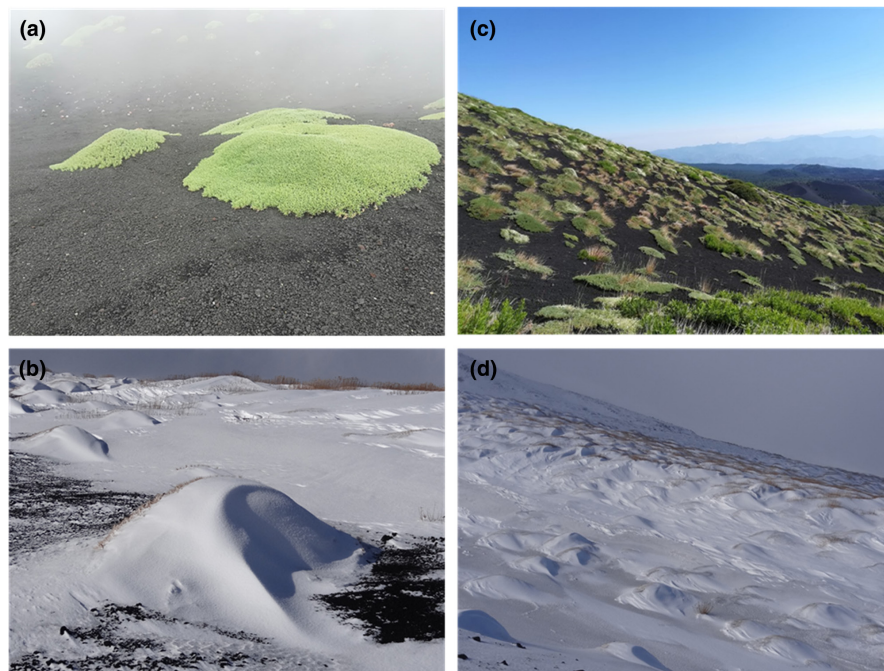


FIGURE 1 Images of *Astragalus siculus* and landscape view of the study site in summer (a–c) and winter (b–d). Pictures by G. Bonanomi and R. Guarino.

the second edition of the Flora of Italy (Pignatti et al., 2019), and the recent checklist of vascular plants of Mt Etna by Sciandrello et al. (2020) was adopted as nomenclatural standard. The collected plant specimens were stored in the Herbarium Austroitalicum (IT, acronym according to Thiers, 2022).

Concerning plant traits, we measured the maximum height and diameter at four orientations of all 45 *Astragalus* cushions. We also measured stem density (i.e., stem number per unit of surface) by counting the number of terminal rosettes in five 20 cm × 20 cm metal squares randomly positioned over each cushion.

2.4 | Soil sampling

Soil was sampled during the growing season (July 2018) using a metal corer. Soil samples (~500 g) were collected from the topsoil (0–20 cm), after removal of the litter layer, in all sampling plots. At each elevation, a total of 30 soil samples were collected inside and outside five *Astragalus* cushions that had already been selected for the vegetation survey, at 1650, 1900, and 2210 m a.s.l. Samples were quickly packed in polyethylene bags, stored at +4°C in a portable refrigerated container, and brought to the labs of the Department of Agricultural Sciences, University of Naples Federico II, for further processing. Microbiological analyses were performed on fresh soil stored at +4°C until the time of DNA extraction (within seven days after collection). Chemical analyses were carried out on air-dried soil at room temperature.

2.5 | Soil physical and chemical analyses

The air-dried soil was sieved (2 mm mesh) and the thin fraction was quantified. Subsequently, the sieved fraction of soil samples (<2 mm)

was analysed for total organic carbon and total nitrogen according to the official Italian methods of soil analysis (Costantini, 2007). Soil organic carbon and total nitrogen were determined by a CNS elemental analyser (Thermo Finnigan, Flash EA 1112). The soil organic C and the total N contents were evaluated in oven-dried (105°C) and finely grounded samples (Fritsch Analysette Spartan 3 Pulverisette 0). The soil C/N ratio was calculated from the organic carbon and total nitrogen data.

2.6 | Soil microbiota and bioinformatics analysis

Before DNA extraction, the soil was sieved to remove coarse impurities and homogenised to avoid bias in differences due to the micro-spatial heterogeneity of the soil. A final representative amount of 500 mg per sample was used for total microbial DNA prepared using the DNeasy Power Soil Kit (Qiagen) according to the manufacturer's instructions.

Soil microbial community diversity and composition were analysed by high-throughput sequencing using the Illumina MiSeq platform. Bacterial diversity was assessed by sequencing the amplified V3–V4 regions of the 16S rRNA gene (~460 bp), while fungal diversity was assessed by sequencing ITS1–2 spacers of the 18S and 28S rRNA genes in eukaryotes (~300 bp). Polymerase chain reaction (PCR) was performed using primers S-D-Bact-0341-b- S-17/S-D-Bact-0785-a-A-21 for bacteria (Berni Canani et al., 2017) and BITS1fw/B58S3-ITS2rev for fungi (Bokulich & Mills, 2013) under the conditions indicated in the original studies. The raw data obtained were filtered and analysed using the DADA2 pipeline in R (Callahan et al., 2016). Taxonomic assignment was performed by aligning sequences in the Greengenes and UNITE v.8 databases (De Santis et al., 2006; Nilsson et al., 2019) for bacterial



and fungal communities respectively. The obtained ASV (amplicon sequence variants) tables were then visually inspected for chloroplast and Streptophyta contamination. Raw sequences are available at the Sequence Read Archive (SRA) of the National Centre for Biotechnology Information (NCBI), under accession number PRJNA894983.

2.7 | Above- and below-ground microclimate

Air temperature, soil temperature, and soil moisture were monitored continuously for 10 consecutive months (from June 2018 to March 2019) with monitoring stations placed both inside and outside the canopy of *Astragalus*. Each station was equipped with an Em 50 data logger, four soil moisture and temperature sensors (5TM), and one air temperature and relative humidity sensor (VP3, Decagon, Pullman, WA, USA). The air temperature and humidity sensors were protected from direct sunlight by a white shield. The probes for soil moisture and temperature were buried in the soil at a depth of -15 cm. The data loggers were programmed to record and store data every 60 min throughout the monitoring period. Unfortunately, the station at 1600 m was damaged by vandalism and no data were recorded.

2.8 | Data analysis

Prior to statistical tests to evaluate significant differences in survey design, data set for cushion height, density, soil chemical parameters, and species richness was tested for homogeneity of variance using Levene's test. Data with heterogeneous variance were then tested using non-parametric methods. In detail, to test the significance of differences in height and density of *Astragalus* cushions across elevation gradients, data were analysed using one-way analysis of variance (ANOVA) and specific differences between elevation points were tested by Duncan's post-hoc test with a significant p -value of less than 0.05.

Soil chemical parameters were then analysed by comparing soil inside and outside the *Astragalus* cushions along the elevation gradient using the two-way ANOVA and Duncan's post-hoc tests. Soil organic carbon and nitrogen did not meet the assumption of homogeneity of variance to conduct a parametric test and were then analysed using a non-parametric methodology. Kruskal-Wallis ANOVA between multiple independent groups and Mann-Whitney U test for pairwise comparison between groups were used.

Soil temperature and moisture data were analysed for differences inside and outside the cushions using log-log plots. Species richness and Shannon index (S and H') were calculated to assess variation in plant alpha diversity within and outside the *Astragalus* cushions and at different elevations. Plant species richness data were then tested for significant changes using one-way ANOVA and Duncan's post-hoc test. The species richness and plant cover data were then used

to calculate the RII (relative interaction index), a symmetric index widely used in plant ecology (Armas et al., 2004). Negative values of RII indicate competitive interaction while positive values indicate facilitation by *Astragalus*. In this case, we calculated the RII to quantify the effect of nurse plant (i.e., *Astragalus* cushions) according to the following equation:

$$\text{RII neighbours} = \frac{(r \text{ inside} - r \text{ outside})}{(r \text{ inside} + r \text{ outside})},$$

where r inside and r outside are the response (either plant cover or species richness) with and without nurse plant (i.e., inside and outside respectively). Two-tailed t tests were used to determine whether RII values were significantly different from zero (at $p < 0.05$).

To assess differences in plant community composition along the elevation gradient and position with respect to cushion, Hellinger-transformed data were used to calculate the resemblance matrix based on the Bray-Curtis distance. The data transformation was used to downweight the importance of prevalent species and increase the role of less dominant species to assess similarity between samples (Clarke & Gorley, 2015). Resemblance data were ordered using metric multidimensional scaling (MDS), and their centroid differences were calculated using bootstrap averaging based on repeated resampling (with replacement, 50 iterations) from the original dataset. The average values were then visualized in a MDS using as many dimensions as needed to closely match the original distance matrix (correlation coefficient of $\rho = 0.99$). The fit of the MDS ordinations was quantified by a stress value.

For microbial species, diversity metrics such as the number of ASVs, Shannon diversity index, and the number of reads of microbial communities in different areas of survey and elevation were calculated. A resemblance matrix calculated using the Bray-Curtis similarity distance was used to perform metric MDS. Significant changes in beta diversity in both plant and microbial data were tested by PERMANOVA (999 permutations) using sampling position with respect to the cushion, that is, inside versus outside, and elevation as fixed factors and cushion identity (sequential number for each cushion) as a random factor to determine if changes in community data were due to conditions specific to each cushion. The p -value was corrected by the Monte Carlo method to avoid bias due to multiple comparisons. For bacteria and fungi, PERMANOVA was performed at the ASV level. Instead, PERMANOVA was performed for plant community data at the species level and by plant life form.

To observe the specific association of taxa groups inside and outside the *Astragalus* cushion and at different elevations, heatmaps were made with the 70 most abundant species for the bacterial and fungal communities. Taxa were ordered by hierarchical clustering according to an index of association similarity.

Multivariate data analysis and multivariate significance tests were performed using Primer 7 software (Clarke & Gorley, 2015) on Hellinger-transformed matrices. Levene's test, and univariate significance tests, both parametric and non-parametric, were performed using Statistica 10 (StatSoft®).

3 | RESULTS

3.1 | *Astragalus* traits and biogenic effects on soil and microclimate

The maximum height of the cushion was significantly higher at 2210 and 1900 m than for plants at 1650 m ($F=8.4$; $p<0.000$). Stem density significantly increased with elevation ($F=16.9$; $p<0.000$) indicating high compactness of the cushion at 2210 m (Figure 2).

Soil parameters inside the *Astragalus* cushions differed significantly from those of outside with elevation and sampling position (inside vs outside) that were statistically significant (Appendix S2). In particular, the thin soil fraction (<2 mm) was similar inside and outside the cushions at 1650 m, but significantly higher inside *Astragalus* at 1900 and 2210 m (Figure 3). Soil organic carbon and total nitrogen content was higher inside the cushions than outside at all elevations (Figure 3; Appendix S2). However, the organic carbon content at low elevation inside the cushion was only 2.62 times higher than outside, while the ratio at 1900 and 2210 m a.s.l. was 12.56 and 11.44 times higher respectively. The total nitrogen content of the soil was 2.97 times higher inside the cushion than outside at low elevation,

but 23.82 times higher at 1900 m a.s.l. and 59.39 times higher at 2210 m a.s.l. than inside the cushion.

The microclimate in the cushions and the soil moisture show a strong seasonality, typical of Mediterranean mountain regions, with soil temperatures ranging from -7.5 to $+41.4^{\circ}\text{C}$. The *Astragalus* cushions had a remarkable biogenic buffering effect on soil temperature at both 1900 and 2210 m a.s.l. (Figure 4a,b). During the growing season, temperatures inside and outside the cushion showed smaller variations, ranging from $+0$ to $+20^{\circ}\text{C}$ at 1900 and from $+0$ to $+15^{\circ}\text{C}$ at 2210 m a.s.l. Specifically, the maximum ground temperature at 2210 m a.s.l. outside the cushion was $+41.4^{\circ}\text{C}$ and inside was only 20.2°C . At 1900 m, the buffer effect was present, but only to a limited extent, as the highest temperature exceeded the inside temperature by only 4.5°C . In contrast, in winter, the temperature inside the cushion was higher than outside, but only when the values dropped below zero (Figure 4a,b). More specifically, the temperature inside the cushion at 1900 m elevation never dropped below $+0^{\circ}\text{C}$ (absolute minima of $+2.1^{\circ}\text{C}$), while outside temperatures often dropped below zero during the winter nights, with a minimum value of -4.10°C . At high elevation (2210 m), soil moisture inside the cushions was higher than outside (Figure 4d). At 1900 m, we found that when the soil was dry (value

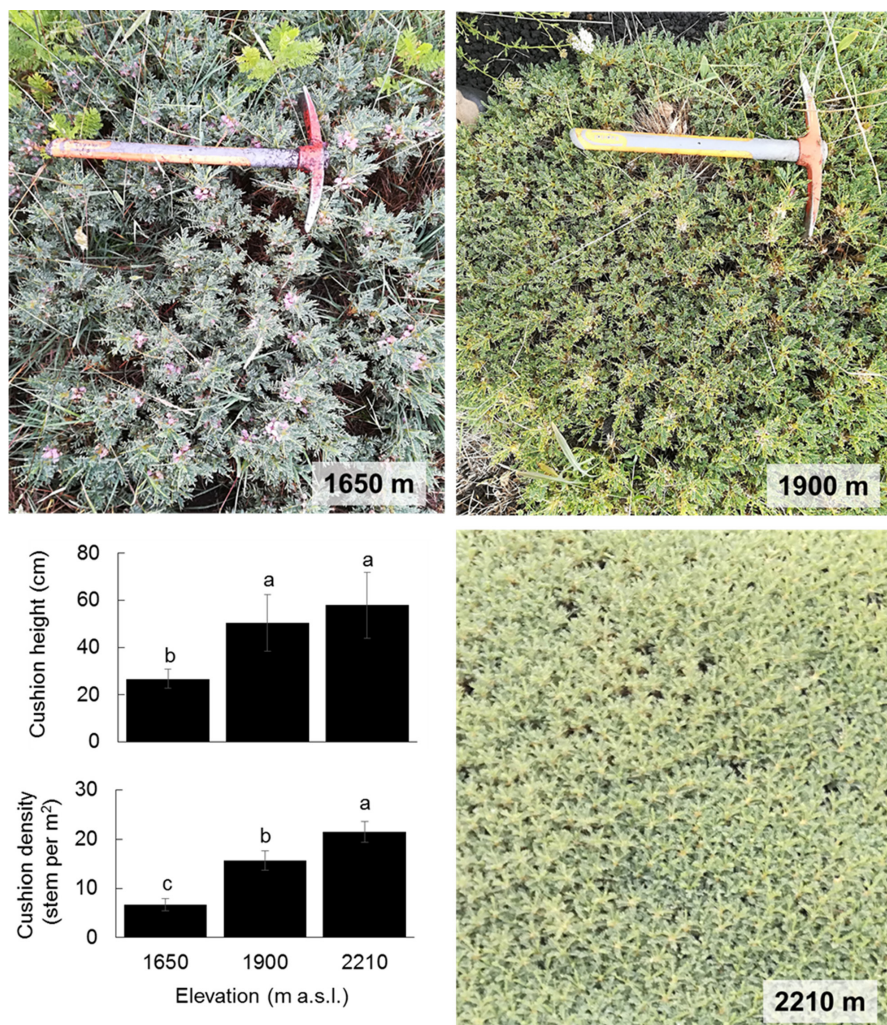


FIGURE 2 Close-up images of *Astragalus siculus* canopy at 1650, 1900 and 2210 m a.s.l. showing difference in stem density along the elevation gradient on the northeastern flank of Mt Etna. Inset reports cushion height and density, in terms of number of stems per square meter. Values are average \pm standard deviation; different letters indicate significant differences at $p<0.05$. Pictures by G. Bonanomi.

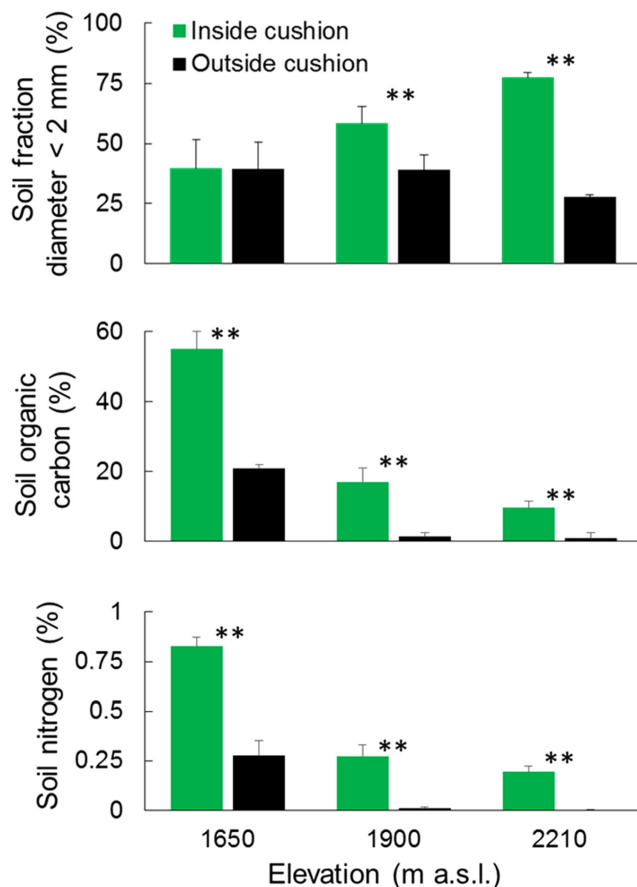


FIGURE 3 Bar plot of soil fraction below 2 mm, organic carbon and total nitrogen inside and outside the cushion plants of *Astragalus siculus* at 1650, 1900 and 2210 m a.s.l., along the northeastern flank of Mt Etna. Error bars refer to standard deviations, asterisks indicate values with significant ($p < 0.01$) differences (Student's *t* test).

below ~0.15%), the moisture content was higher inside the cushions, but above this threshold, soil moisture was higher outside the cushions (Figure 4c).

3.2 | Plant–plant interaction and community structure and diversity

A total of 28 vascular plants were recorded at the study site (Appendix S3). The RII showed negative values at low elevation (1650 m) but shifted to positive at medium (1900 m) and high (2210 m) elevation. The same behaviour was observed for both plant cover and species richness (Table 1). Notably, RII values were more positive at 1900 m than at 2210 m for both plant cover and species richness.

The first MDS axis separated the vegetation sampled inside and outside the cushions, while the second axis concerned the elevation of the study sites (Figure 5). Notably, the effect of elevation was more pronounced for the samples from outside, which were clearly separated at 1650, 1900, and 2210 m a.s.l. In contrast, vegetation inside

the cushions was less differentiated with respect to elevation, with substantial overlap in vegetation sampled at 1900 and 2210 m a.s.l. At the species level, *Astragalus* facilitate many more species at 1900 and 2210 m a.s.l. than at 1650 m a.s.l. (Appendix S10). Specifically, no plant species was associated with the cushion at 1650 m, but we found seven species positively associated with the cushion at 1900 m (i.e., *Bellardiocloa variegata* subsp. *aetnensis*, *Cerastium tomentosum*, *Cuscuta epithymum* L. subsp. *kotschyi* (Des Moul.) Arcang., *Festuca circummediterranea* Patzke, *Galium aetnicum*, *Silene italica* subsp. *sicula*, and *Tanacetum vulgare* subsp. *siculum*), and four at 2210 m (i.e., *Cuscuta epithymum* subsp. *kotschii*, *Galium aetnicum*, *Secale strictum* C. Presl subsp. *strictum*, and *Robertia taraxacoides*). Some plant species were strongly associated with the area outside the cushion even at high elevation, such as *Saponaria sicula* Raf. and *Rumex scutatus* subsp. *aetnensis* (Appendix S10).

3.3 | Bacterial and fungal microbiota inside and outside *Astragalus* cushions

From a microbial diversity perspective, the presence of *Astragalus* cushions impacts the bacterial and fungal microbiome compared to the environment outside the plant canopy (Appendix S4). However, the effect of the cushions is not systematically present at all elevations where we tested them but varies and is more evident at 2210 m (Appendix S5). Specifically, no significant differences were found for the prokaryotic community between inside and outside of the cushions in terms of species richness (*S*). Regarding the number of reads, significant differences were observed between inside and outside at 1900 and 2210 m elevation with the community outside the cushion showing higher values. Similar to the species richness values, the Shannon index also showed a decrease in value with elevation, with significant differences only at the 1900 m elevation, where the bacterial community inside the cushion is richer. The fungal community showed different trends in species richness and number of reads that increase with elevation, while the differences between inside and outside decreased significantly at 1900 m, where a higher number of reads was observed outside the cushions. The Shannon index of the fungal community was higher outside the cushion only at 2210 m elevation (Appendix S4).

At the phylum level, the prokaryotic microbiota consists of a higher percentage of Actinobacteriota, followed by Proteobacteria, Planctomycetota, Acidobacteriota, Gemmatimonadota, and Chloroflexi at 1650 m (Figure 6). At 1900 m, the dominance of Actinomycetota decreases and gives way to Proteobacteria, which become the dominant phylum, and Planctomycetota and Gemmatimonadota, which increase in relative abundance. At 2210 m the community is dominated by Actinobacteriota and Proteobacteria, which have similar relative abundance, with other taxa contributing equally to community structure (Figure 6). When comparing the change in bacterial community with elevation within the cushion, no significant changes are observed, while significant

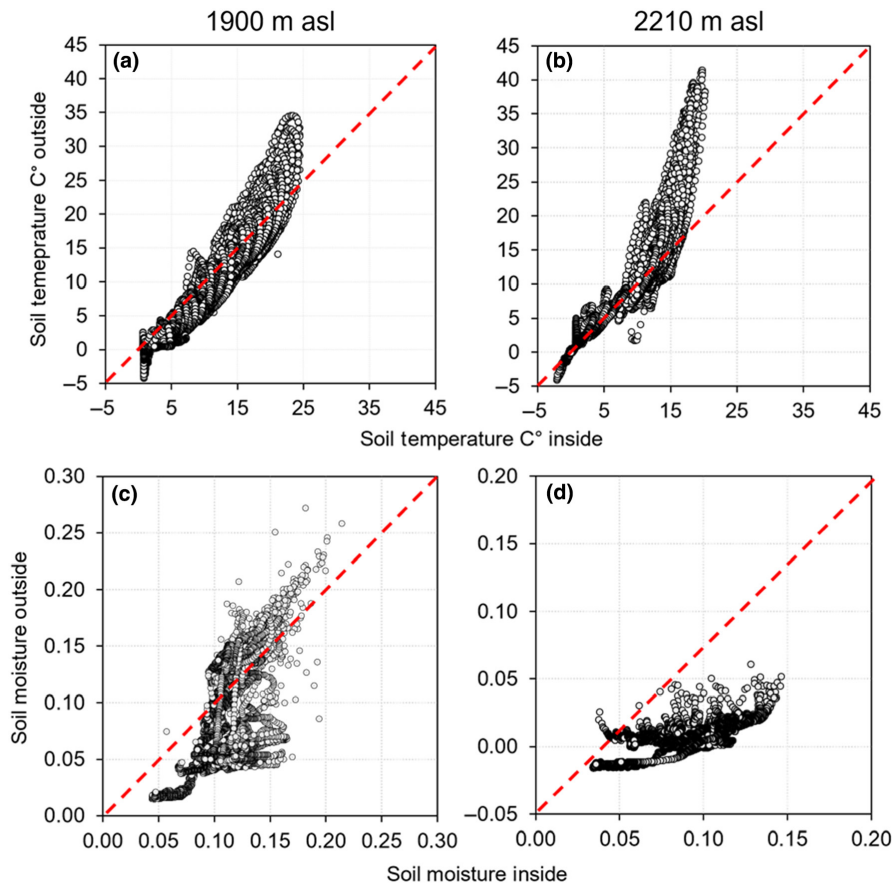


FIGURE 4 Scatter plot of soil temperature ($^{\circ}\text{C}$) and moisture (%) recorded outside (OUT) and inside (IN) cushions of *Astragalus siculus* at 1900 and 2210 m a.s.l. Data were recorded in a 10-months period from June 2018 to March 2019.

changes are observed in community composition outside the cushion between 1650 and 2210 m a.s.l. (1650 vs 2210, $t=28.896$, $p=0.013$) (Appendix S6).

At the phylum level (Appendix S7), at 1650 m the prokaryotic community inside the cushion harbours a higher abundance of Proteobacteria, Actinobacteriota, and to a lesser extent Myxococcota, Bacteroidota, and Gemmatimonadota. The taxa more abundant outside the cushion belong to Chloroflexi, Acidobacteriota, and Firmicutes (Appendix S7). At 1900 m, the same phyla escape to the interior of cushion plants with additional contributions from Planctomycetota and Bacteroidota and exclusion of Actinobacteriota. Similarly, at high altitude, 2210 m, Actinobacteriota, Bacteroidota, Planctomycetota, and Verrucomicrobiota increase in abundance inside the cushions, while Gemmatimonadota are displaced outside the cushion. The changes between inside and outside the cushion are particularly evident at 1900 m ($t=23.095$, $p=0.030$) and at 2210 m ($t=31.214$, $p=0.011$) (Appendix S6).

At 1650 m, *Streptomyces*, *Kribbella*, *Jatrophihabitans*, Solirubrobacterales, and *Gemmatimonas* were more abundant inside the cushion while *Crossiella*, *Bryobacter*, *Amycolatopsis*, and IMCC26256 were more abundant outside. At 1900 m, a more distinct association of bacterial genera is observed inside the cushions, with the genera *Burkholderia*, *Sphingomonas*, *Gemmatimonas*, and *Mycobacterium*, while the ASVs of Acetobacteraceae, IMCC26256, *Granulicella*, *Crossiella*, and *Dyella* show a higher relative contribution outside. At 2210 m, the differentiation/association between

inside and outside the cushions became stronger (Appendices S6 and S8), with genera of *Sphingomonas*, *Bryobacter*, *Nakamurella*, *Mycobacterium*, Chitinophagaceae, and *Jatrophihabitans* thrived inside the cushion, while Gemmatimonadaceae, Acetobacteraceae, *Dyella*, Oxalobacteraceae, Frankiales, IMCC26256, *Psseudonocardia*, and *Tundrisphaera* were preferentially found outside (Figure 7).

Mycobiota showed a change in relative abundance with elevation. At 1650 m, the community consists mainly of fungi of the phylum Ascomycota, followed by Basidiomycota and Mortierellomycota. With increasing elevation, the abundance within the community remains similar, being dominated by Ascomycota, followed by Basidiomycota and Mortierellomycota (Figure 7). The comparison of the fungal microbiota inside the cushions did not change with elevation, while the difference in composition outside the cushion was evident between 1650 and 2210 m ($t=22.508$, $p=0.032$) and between 1900 and 2250 m ($t=19.997$, $p=0.043$) (Appendix S5). When considering the changes between inside and outside the cushion at each elevation, no difference was observed at 1650 m (Appendix S7), while at 1900 and 2250 m there was a drastic increase in Basidiomycota and Mortierellomycota (Appendix S7). Phylum-level results were also confirmed by permutational multivariate ANOVA (PERMANOVA) analysis at the lowest taxonomic level, with significant differences comparing microbiota inside and outside the cushion at 1900 m ($t=21.642$, $p=0.036$) and 2210 m ($t=25.920$, $p=0.011$) (Appendices S6 and S8). Specific associations for ASVs were also observed in fungi identified at the lowest

TABLE 1 Relative Interaction Index (RII) for plant cover, species richness and plant species calculated at 1650, 1900 and 2210 m a.s.l.

	Elevation (m a.s.l.)		
	1650	1900	2210
Plant cover	-0.23	0.49	0.13
Species richness	-0.51	0.57	0.08
<i>Achillea ligustica</i>	-0.91	—	—
<i>Bellardiochloa variegata</i>	—	0.54	0.11
<i>Calamagrostis epigejos</i>	-0.24	—	—
<i>Centaurea giardinae</i>	-0.42	—	—
<i>Cerastium tomentosum</i>	—	1	—
<i>Chondrilla juncea</i>	-0.49	—	—
<i>Crepis leontodontoides</i>	-0.25	—	—
<i>Cuscuta epithymum</i>	—	1	1
<i>Festuca circummediterranea</i>	-0.46	1	—
<i>Galium aetnicum</i>	0.19	0.53	1
<i>Jasione montana</i>	-1	—	—
<i>Lactuca viminea</i>	0.17	—	—
<i>Phleum hirsutum</i>	-0.07	—	—
<i>Pteridium aquilinum</i>	-1	—	—
<i>Robertia taraxacoides</i>	—	-0.09	0.35
<i>Rumex scutatus</i>	-1	-0.51	-0.37
<i>Saponaria sicula</i>	—	—	-1
<i>Secale strictum</i>	0.22	0.28	1
<i>Senecio squalidus</i>	—	-1	0.04
<i>Silene italica</i>	-0.83	1	—
<i>Tanacetum vulgare</i> subsp. <i>siculum</i>	-0.09	0.93	0.01
<i>Tragopogon crocifolius</i> subsp. <i>nebrodensis</i>	-0.41	—	—

Note: Values in bold indicate significant RII values obtained from *t* tests ($p < 0.05$). Negative values of RII indicate competitive interaction while positive values indicate facilitation by *Astragalus siculus*.

taxonomic level (Appendix S9). At 1650 m inside the cushion the community is characterized by the presence of *Aspergillus flavus* Link, *Penicillium roseopurpureum* R.P.Dierckx, and an ASV belonging to the Agaricomycetes. Instead, *Curvularia*, *Mortierella alpina* Peyronel, *Calyptrozyma*, *Clitopilopsis hirneola* (Fr.) Kühner and *Penicillium subrubescens* Houbraken, Mansouri & Frisvad are more abundant outside the cushion. At 1900 m, the differentiation of the fungal community is more evident with *Hydnomerulius pinastri* (Fr.) Jarosch & Besl, Stephanosporaceae, *Mortierella lignicola* (G.W.Martin) W.Gams & R.Moreau, and fungi of the genus *Chalara* more abundant inside the cushion, while *Myriospora smaragdula* (Wahlenb.) Nägeli, *Penicillium subrubescens* Houbraken, Mansouri & Frisvad, and an ASV of the family Eremomycetaceae are more abundant outside. At 2210 m elevation, the soil inside the cushions is dominated by *Hydnomerulius pinastri* (Fr.) Jarosch & Besl, *Trechispora stellulata* (Bourdot & Galzin)

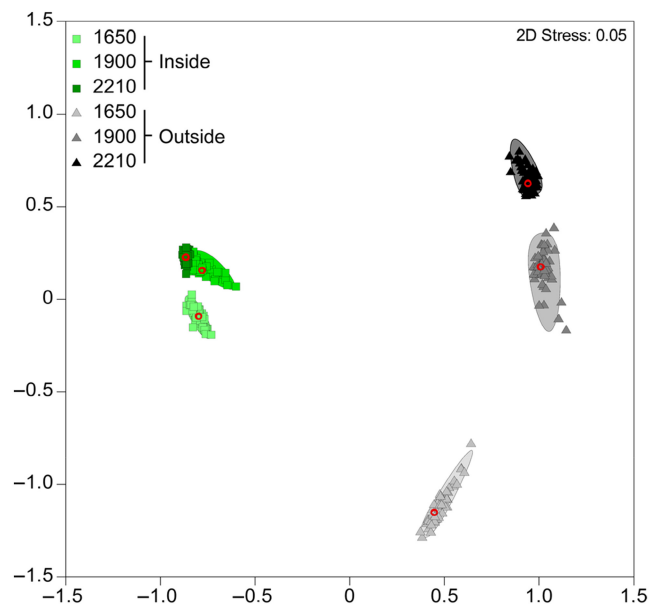


FIGURE 5 Multidimensional scaling (MDS) plot of bootstrap averages (50 repetitions) of plant community on the inside (squares) and outside (triangles) of 90 plots of the cushion plant *Astragalus siculus* at three different elevations (1650; 1900 and 2210 m a.s.l.) from Mt Etna based on Bray–Curtis similarity of square-root-transformed data. Individual repetitions are based on random draw and replacement of samples from the original data set. Red circles represent the overall centroids across all repetitions. Boundary lines represent 95% confidence regions. Goodness of representation of communities is expressed as stress values according to Clarke & Gorley, 2015.

Liberta, and *Elasticomyces elasticus* Zucconi & Selbmann. Conversely, outside, *Myriospora smaragdula* and *Pseudogynoaascus roseus* Rallo are the most abundant species (Figure 7).

4 | DISCUSSION

With increasing elevations, *Astragalus siculus* forms compact, dome-shaped cushions that provide shelter, microclimate, and resource patches for other organisms. It can also trap moisture, nutrients, and organic matter, creating more favourable conditions for other organisms to grow and survive (Pignatti et al., 1980). Therefore, cushion-forming *Astragalus siculus* can be considered an ecosystem engineer, as it creates physical structures that have significant effects on the biotic and abiotic components of the surrounding environment. Some of these effects will be examined in the following paragraphs.

4.1 | *Astragalus* biogenic effects on above- and below-ground environments

Cushion-forming species can buffer the temperature regime and moisture under the canopy thanks to their domed shape and dense canopy. Previous studies reported an increase in canopy

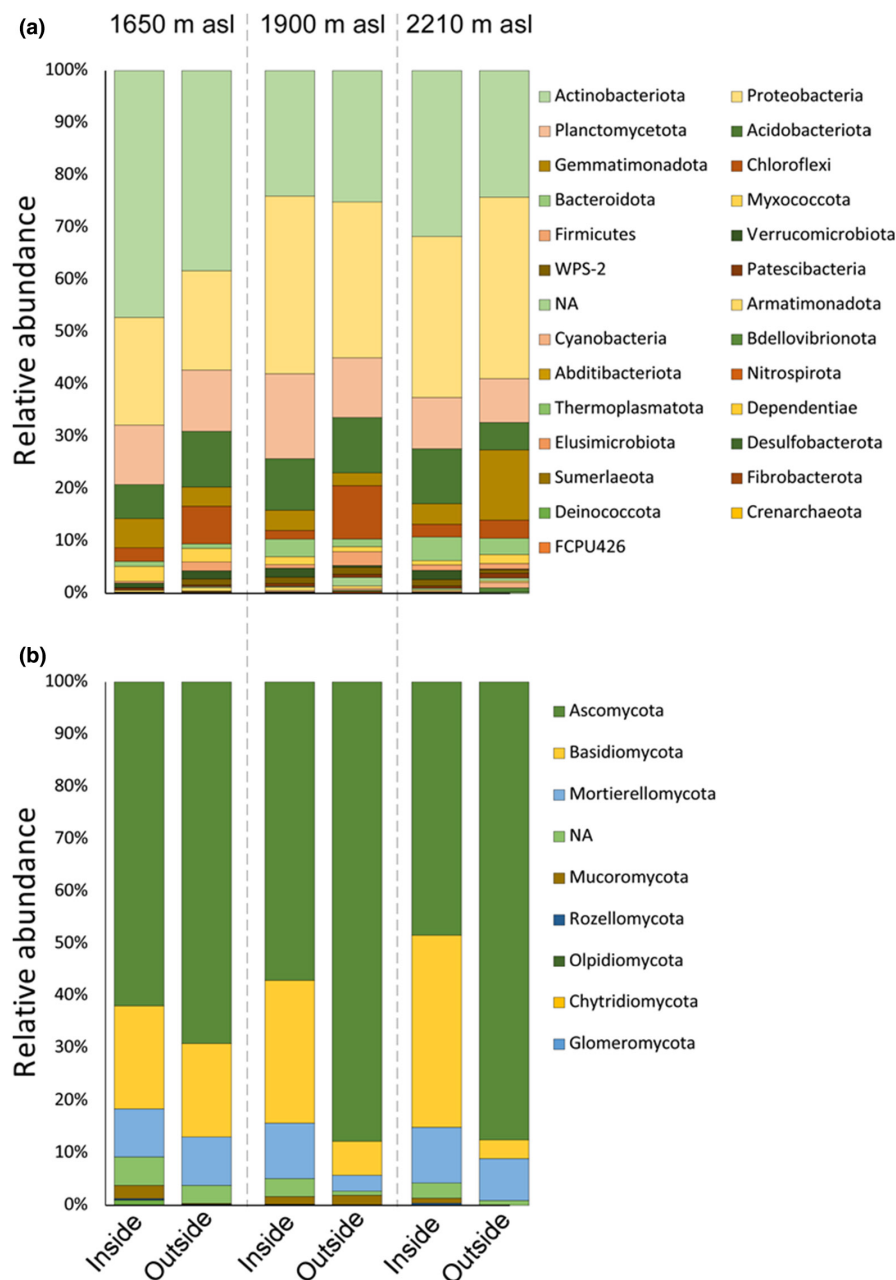


FIGURE 6 Stacked bar plot for bacteria (above, a) and fungi (below, b) showing relative abundance of different phyla inside and outside cushion of *Astragalus sicularis* at 1650, 1900 and 2210 m a.s.l. along the northeastern flank of Mt Etna.

temperature for cushion-forming plants such as *Silene acaulis* subsp. *bryoides* (Jord.) Nyman (Bonanomi et al., 2016), *Azorella monantha* Clos (Cavieres et al., 2005), and *Laretia acaulis* Gillies & Hook. (Cavieres et al., 2006). The canopy of all these species is very dense and compact and is capable of storing litter, organic material, and also peat between shoots (Aubert et al., 1914), which acts as an efficient heat and moisture trap. We found that temperatures inside the canopy were cooler than outside when temperatures were above the threshold of $\sim 20^{\circ}\text{C}$, but higher when temperatures outside the cushion fell below zero. The retention of snowpack inside the canopy compared to the adjacent open field may help explain the buffering effect for the lowest winter temperatures. Overall, the *Astragalus* canopy acts as a buffering system, reducing high temperatures and increasing low temperatures compared to bare ground. The cooling effect of the soil surface is especially evident in volcanic soil, which

is very dark (Figure 1) and can reach surface temperatures above 80°C on sunny, summer days also at high elevation, as reported for the volcanic southern slope of Mt Vesuvio (Stinca et al., 2015) and the eastern slope of Stromboli (Richter, 1984). In terms of microclimate, the canopy of *Astragalus* is functionally more similar to that of shrubs and trees (De Frenne et al., 2019; Rita et al., 2021) than of other cushion species. *Astragalus* forms semi-compact, creeping cushions according to the classification of Aubert et al. (1914). Although canopy stem density increases with elevation, a pattern previously reported for other cushion species (Hedberg, 1964; Schöb et al., 2013), cushions of *Astragalus* at higher elevations never reach a density and compactness similar to those reported for *Silene acaulis* (Bonanomi et al., 2016).

The increasing stem density of *Astragalus* with elevation probably contributes to the accumulation of fine soil particles within the

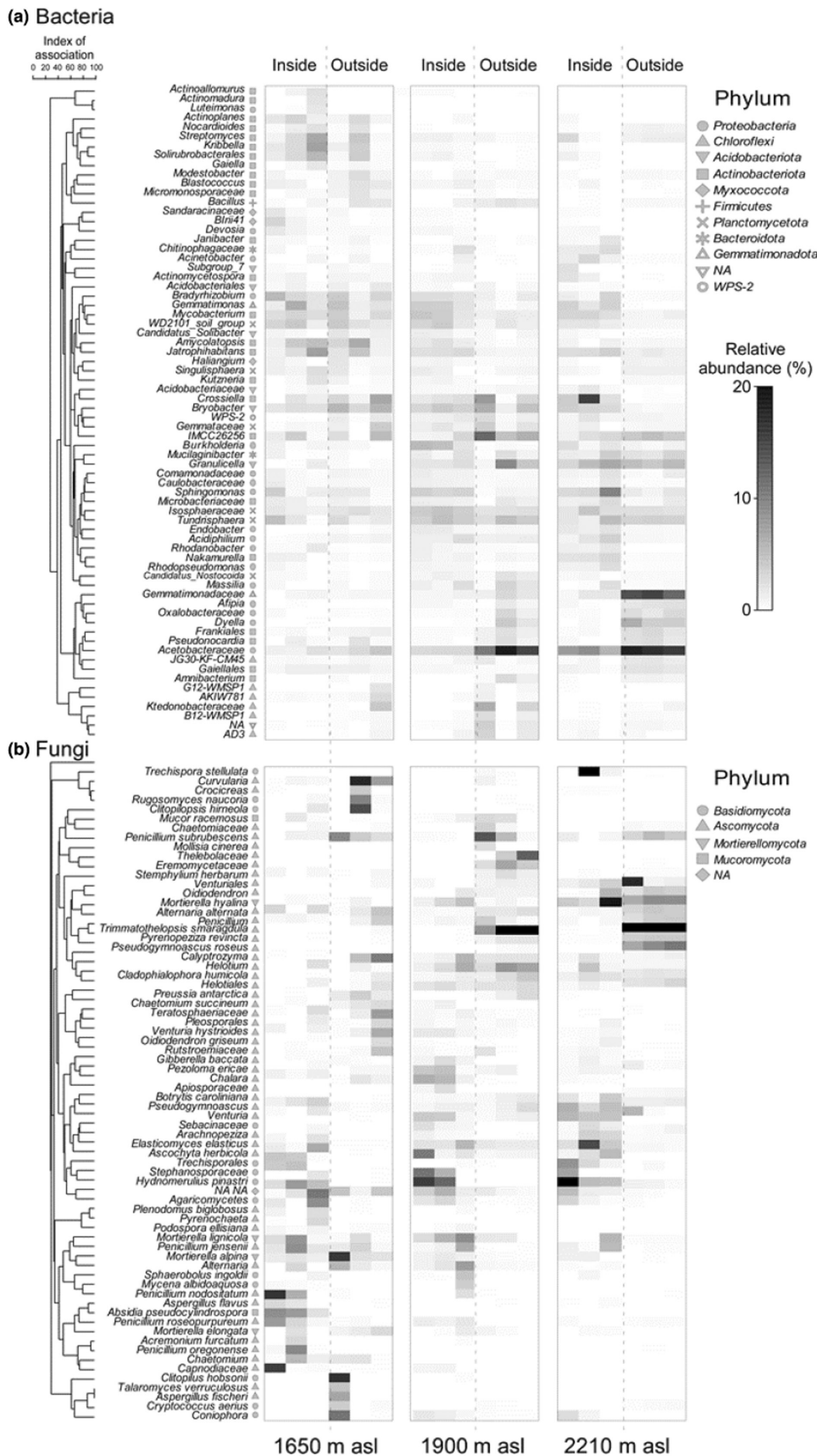


FIGURE 7 Heatplots for bacteria (above) and fungi (below) showing the relative abundance of 70 most abundant OUTs inside and outside cushions of *Astragalus siculus* and at 1650, 1900 and 2210 m a.s.l. along the northeastern flank of Mt Etna. Symbols indicate microbial phyla; microbial taxa are ordered according to hierarchical clustering based on index of association.

canopy that would otherwise be swept away by the periodic strong winds. Accumulation of fine soil particles is an important step in the formation of fertility islands and has been described for both deserts (Wezel et al., 2000) and alpine plants (Körner, 2021). However, *Astragalus* accumulates not only fine particles in the canopy, but also a very high concentration of organic carbon and nitrogen. Thus, total nitrogen was only 2.97 times higher inside the cushion at 1650m than outside, but 23.82 and 59.39 times higher at 1900 and 2210m, respectively. Most of the studies to date have been on non-nitrogen-fixing cushion plants (Aubert et al., 1914), with no specific studies on cushion plants belonging to the Fabaceae. Since *Astragalus* is a nitrogen-fixing plant, it has tissues with high nitrogen content that, once decomposed, would contribute to the formation of the large nitrogen stock under the canopy, as has been reported for several shrubs and trees (Facelli & Brock, 2000; Stinca et al., 2015). Notably, the effects of *Astragalus* canopy on soil fertility were more pronounced at 1900 and 2210m, where the outside soil is rich in gravel and extremely low in nitrogen. Future studies will elucidate the relationship between fertility island establishment and ontogenetic development of *Astragalus*, as has been reported for other nurse species such as *Acacia papyrocarpa* Benth. (Facelli & Brock, 2000), *Genista etnensis* (Raf.) DC. (Stinca et al., 2015), and *Juniperus communis* L. (Allegrezza et al., 2016).

4.2 | Interaction of *Astragalus* with co-existing vascular plants

Cushion plants are known for their facilitative effects in alpine environments in numerous mountainous regions (Reid et al., 2010). Consistent with previous studies (Cranston et al., 2012), we found that *Astragalus* cushions promoted several heterospecifics, including annual and perennial forbs and grasses. At the community level, we found that interactions between *Astragalus* and plants from 1650 to 1900 and 2210m of elevation changed from neutral or negative to positive for several species. The magnitude of the interaction effect, expressed by the RII, was higher at 1900m, resulting in a hump-shaped facilitation curve. This result contrasts with previous data reporting a monotonic increase in facilitation with elevation for *Silene acaulis* (Antonsson et al., 2009; Cranston et al., 2012) and other alpine cushion plants in the Andes (Cavieres et al., 2002, 2005). However, in the above studies, no information was provided on the amplitude of the altitudinal range of the cushion plant, and the ranges studied were shorter (300m in Antonsson et al., 2009; 243m in Cranston et al., 2012; 200m in Cavieres et al., 2002 and 2005) than those of our study (650m). Moreover, our results are consistent with the study of *Silene acaulis*, which reported that along a 700m elevation transect the greatest facilitation effect occurred at mid-elevation (Bonanomi et al., 2016). These results underscore the importance of exploring the full length of the severity gradient to properly determine the shape of the competitive and facilitative interaction (Le Roux & McGeoch, 2010) as well as the realized niche of a target species (Marcenò et al., 2022).

The SGH model predicts a linear increase in the intensity of facilitative interaction as environmental conditions become harsher (Bertness & Callaway, 1994). However, the observed hump-shaped facilitation effect suggests that the SGH model should also consider that abiotic stresses may filter local species pools and reduce resource availability, resulting in weaker facilitation effects at higher elevations. Our results support an alternative theoretical model, in which the net positive interaction decreases or collapses at the upper extreme of the abiotic stress gradient (Michalet et al., 2006; Maestre et al., 2009; Holmgren & Scheffer, 2010). For *Silene acaulis*, Bonanomi et al. (2016) explained the breakdown of facilitation at the upper end of the elevation gradient by changes in the morphological characteristics of the cushion plant, which become so dense and compact that it limits its nurse capability at the expense of co-existing plants. Such findings link shifts in plant functional traits along environmental gradients to interactions between plants (Schöb et al., 2013). In the case of *Astragalus*, our study shows that plants rooted inside the cushion would benefit from a buffered temperature regime, a wetter and more nutrient-rich soil, and a different bacterial and fungal microbiota. It is noteworthy that all of these effects come into play the most at 1900 and 2210m of elevation. However, our study cannot disentangle the role and importance of the different mechanisms of facilitation. Future studies aimed at understanding the weight of the different facilitation mechanisms, also in relationship to the size of the host cushion and to the environmental filtering acting on the life cycle of co-existing plants as elevation increases, will be useful to understand why positive interactions are reduced at higher elevations.

4.3 | *Astragalus* modulates soil bacterial and fungal microbiota

The effect of the cushions on the soil microbiota is more pronounced at 1900 and 2210m elevation, probably due to the biogenic ability of the plant to modify the microhabitat under the canopy. In contrast, at 1650m elevation, similar abiotic conditions inside and outside the cushion favour relatively similar microbial communities. Outside the cushion, the condition of the microbial community changes with increasing elevation probably because the environment is poor in organic matter and total nitrogen and is subject to significant temperature and moisture fluctuations. The most representative taxon in the fungal community is the lichen *Myriospora smaragdula*, which is well adapted to an exposed environment characterised by harsh conditions and adopts a photolithoautotrophic strategy for survival (Purvis et al., 2000; Rosa et al., 2020). The rocky and harsh characteristics of the soil outside the cushions are also reflected in the presence of psychrotolerant Ascomycota of the genus *Pseudogymnoascus* (Villanueva et al., 2021) and prokaryotic organisms such as those of the genus *Dyella* (Wang et al., 2021), which are typical of rocky environments, bacteria of the genus *Crossiella* (Labeda, 2015), which are more abundant in cold environments, and *Tundrisphaera*, which are psychrotolerant and associated with lichens (Kulichevskaya

et al., 2017). Inside the cushions, the most obvious difference in the microbiome community structure is the high abundance of Basidiomycota. The high content of organic matter and nitrogen makes each cushion a microsite with high resource concentration and biological activity. Indeed, taxa of the Basidiomycota such as *Hydnomerulius pinastri* and Stephanosporaceae are decomposers of leaf litter and woody debris (Binder & Hibbett, 2006; Cannon & Kirk, 2007; Sista Kameshwar & Qin, 2020), with *Trechispora stellulata* capable of forming ectomycorrhizal symbiosis (Vanegas-León et al., 2019). The island of fertility created by the cushion also hosts other microbial actors and possibly other potential relationships. For example, *Mortierella lignicola* is one of the most abundant mycetes normally associated with woody debris in termite nests. Interestingly, bacteria of the phylum Bacteroidota also proliferate under the canopy and are often associated with termite guts in a three-way symbiosis (Otani et al., 2014).

At 2210m, *Elasticomyces elasticus* is one of the most common fungi associated with the cushion and is usually isolated in psychrophilic and oligotrophic environments associated to rocks, sphagnum and lichens (Selbmann et al., 2008). Contrary to what was observed by Venice et al. (2023) on *Astragalus nebrodensis* (Guss.) Strobl, no evidence of association with plant symbionts in the soil, such as arbuscular mycorrhizal fungi or nitrogen-fixing bacteria, was observed, which appear to be present but vary little with respect to the soil outside the cushion. This result is consistent with the findings of Cáceres-Mago et al. (2022), who found no consistent effects of the nurse plant *Hypericum laricifolium* Juss. on arbuscular mycorrhizal fungal colonization of the roots of some beneficiary species.

5 | CONCLUSION

In this study, we found that *Astragalus* cushions have strong biogenic effects on above- and below-ground conditions by increasing soil organic carbon and nitrogen stocks, buffering soil temperature and moisture regimes, and harbouring a specific bacterial and fungal microbiota compared to bare soil. As a result of these biogenic environmental changes, the effect of *Astragalus* on some of the co-existing species shifted from negative or null at 1650m to facilitative at 1900 and 2210m of elevation. Furthermore, our data show a relationship between *Astragalus* morphological traits and soil biogenic changes related to soil chemistry and microbiota that affect plant interaction and coexistence. However, our study provides only indirect evidence for facilitation and possible causal mechanisms. Further field experiments, including manipulation of cushion structure along the elevation gradient, would be useful to determine the relative roles of microclimate buffering, soil fertility, and bacterial and fungal microbiota within the *Astragalus* cushion in facilitative interactions.

AUTHOR CONTRIBUTIONS

Giuliano Bonanomi, Riccardo Guarino, Adriano Stinca contributed to the conceptualization and design of the research; Giuliano Bonanomi, Riccardo Guarino, Adriano Stinca, Gian Pietro Giusso

Del Galdo collected the data; Maurizio Zotti and Mohamed Idbella did the molecular analyses; Giulia Maisto and Anna De Marco did the soil analyses; Maurizio Zotti and Mohamed Idbella analysed the data; Giuliano Bonanomi led the writing of the manuscript, with substantial inputs from Riccardo Guarino and Adriano Stinca; all authors served as scientific advisers and critically reviewed the first draft of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in the supplementary online material.

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REFERENCES

- Allegrezza, M., Corti, G., Cocco, S., Pesaresi, S., Chirico, G., Saracino, A. et al. (2016) Microclimate buffering and fertility Island formation during *Juniperus communis* ontogenesis modulate competition-facilitation balance. *Journal of Vegetation Science*, 27, 616–627.
- Anthelme, F., Meneses, R.I., Valero, N.N.H., Pozo, P. & Dangles, O. (2017) Fine nurse variations explain discrepancies in the stress-interaction relationship in alpine regions. *Oikos*, 126, 1173–1183.
- Antonsson, H., Björk, R.G. & Molau, U. (2009) Nurse plant effect of the cushion plant *Silene acaulis* (L.) Jacq. in an alpine environment in the subarctic Scandes, Sweden. *Plant Ecology and Diversity*, 2, 17–25.
- Armas, C., Ordiales, R. & Pugnaire, F.I. (2004) Measuring plant interactions: a new comparative index. *Ecology*, 85(10), 2682–2686.
- Arredondo-Núñez, A., Badano, E. & Bustamante, R. (2009) How beneficial are nurse plants? A meta-analysis of the effects of cushion plants on high-Andean plant communities. *Community Ecology*, 10(1), 1–6.
- Aubert, S., Boucher, F., Lavergne, S., Renaud, J. & Choler, P. (1914) 2014: a revised worldwide catalogue of cushion plants 100 years after Hauri and Schröter. *Alpine Botany*, 124, 59–70.
- Baldrian, P. (2017) Forest microbiome: diversity, complexity and dynamics. *FEMS Microbiology Reviews*, 41(2), 109–130.
- Barbera, G., Cullotta, S. & Pizzurro, G. (2004) Agroforestry systems of Mt Etna, Italy: biodiversity analysis at landscape, stand and specific level. In: Marchetti, M. (Ed.) *Monitoring and indicators of forest biodiversity in Europe—from ideas to operationality*, Vol. 51. Joensuu: EFI Proceedings, pp. 481–492.
- Barot, S., Ugolini, A. & Brikci, F.B. (2007) Nutrient cycling efficiency explains the long-term effect of ecosystem engineers on primary production. *Functional Ecology*, 21, 1–10.
- Bazan, G., Marino, P., Guarino, R., Domina, G. & Schicchi, R. (2015) Bioclimatology and vegetation series in Sicily: a geostatistical approach. *Annali Botanici Fennici*, 52, 1–18. Available from: <https://doi.org/10.5735/085.052.0202>
- Berni Canani, R., De Filippis, F., Nocerino, R., Laiola, M., Paparo, L., Calignano, A. et al. (2017) Specific signatures of the gut

- microbiota and increased levels of butyrate in children treated with fermented cow's milk containing heat-killed *Lactobacillus paracasei* CBA L74. *Applied and Environmental Microbiology*, 83, e01206–e01217.
- Bertness, M.D. & Callaway, R. (1994) Positive interactions in communities. *Trends in Ecology & Evolution*, 9, 191–193.
- Binder, M. & Hibbett, D.S. (2006) Molecular systematics and biological diversification of Boletales. *Mycologia*, 98, 971–981.
- Bokulich, N.A. & Mills, D.A. (2013) Improved selection of internal transcribed spacer-specific primers enables quantitative, ultra-high-throughput profiling of fungal communities. *Applied and Environmental Microbiology*, 79, 2519–2526.
- Bonanomi, G., Stinca, A., Chirico, G.B., Ciaschetti, G., Saracino, A. & Incerti, G. (2016) Cushion plant morphology controls biogenic capability and facilitation effects of *Silene acaulis* along an elevation gradient. *Functional Ecology*, 30, 1216–1226.
- Branca, S., Coltelli, M. & Gropelli, G. (2011) Geological evolution of a complex basaltic stratovolcano: Mount Etna, Italy. *Italian Journal of Geosciences*, 130(3), 306–317.
- Branca, S., Coltelli, M., Gropelli, G. & Lentini, F. (2011) Geological map of Etna volcano, 1: 50,000 scale. *Italian Journal of Geosciences*, 130(3), 265–291.
- Brooker, R.W. & Callaghan, T.V. (1998) The balance between positive and negative plant interactions and its relationship to environmental gradients: a model. *Oikos*, 81, 196–207.
- Brullo, S., Cormaci, A., Giusso del Galdo, G., Guarino, R., Minissale, P. & Spampinato, G. (2005) A syntaxonomical survey of the Sicilian dwarf shrubs vegetation belonging to the class Rumici-Astragaletea siculi. *Annali di Botanica*, 5, 57–104.
- Cáceres-Mago, K., Cáceres, A. & Llambí, L.D. (2022) Effects of nurse shrubs on symbioses between soil fungi and associated plants along a tropical alpine elevation gradient. *Alpine Botany*, 132, 285–300.
- Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A. & Holmes, S.P. (2016) DADA2: high-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13, 581–583.
- Callaway, R.M. (2007) *Positive interactions and interdependence in plant communities*. Berlin: Springer Science & Business Media.
- Cannon, P.F. & Kirk, P.M. (2007) *Fungal families of the world*. Egham, Surrey: Cabi.
- Cavieres, L., Arroyo, M.T., Peñaloza, A., Molina-Montenegro, M. & Torres, C. (2002) Nurse effect of *Bolax gummifera* cushion plants in the alpine vegetation of the Chilean Patagonian Andes. *Journal of Vegetation Science*, 13, 547–554.
- Cavieres, L.A., Badano, E.I., Sierra-Almeida, A., Gómez-González, S. & Molina-Montenegro, M.A. (2006) Positive interactions between alpine plant species and the nurse cushion plant *Laretia acaulis* do not increase with elevation in the Andes of Central Chile. *New Phytologist*, 169(1), 59–69.
- Cavieres, L.A., Quiroz, C.L., Molina-Montenegro, M.A., Muñoz, A.A. & Pauchard, A. (2005) Nurse effect of the native cushion plant *Azorella monantha* on the invasive non-native *Taraxacum officinale* in the high-Andes of Central Chile. *Perspectives in Plant Ecology, Evolution and Systematics*, 7(3), 217–226.
- Clarke, K. & Gorley, R. (2015) Getting started with PRIMER v7. PRIMER-E: Plymouth, Plymouth marine laboratory 20.
- Costantini, E.A.C. (2007) *Metodi di valutazione dei suoli e delle terre, XII+922 pages, with CD-rom*. Siena: Edizioni Cantagalli.
- Cranston, B.H., Callaway, R.M., Monks, A. & Dickinson, K.J. (2012) Gender and abiotic stress affect community-scale intensity of facilitation and its costs. *Journal of Ecology*, 100, 915–922.
- De Frenne, P., Zellweger, F., Rodríguez-Sánchez, F., Scheffers, B.R., Hylander, K., Luoto, M. et al. (2019) Global buffering of temperatures under forest canopies. *Nature Ecology & Evolution*, 3, 744–749.
- De Santis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K. et al. (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*, 72, 5069–5072.
- Dini-Andreote, F. & Raaijmakers, J.M. (2018) Embracing community ecology in plant microbiome research. *Trends in Plant Science*, 23(6), 467–469.
- Facelli, J.M. & Brock, D.J. (2000) Patch dynamics in arid lands: localized effects of *Acacia papyrocarpa* on soils and vegetation of open woodlands of South Australia. *Ecography*, 23(4), 479–491.
- Guarino, R., Giusso Del Galdo, G. & Pignatti, S. (2006) The Mediterranean dwarf shrubs: origin and adaptive radiation. *Annali di Botanica*, 5, 93–101.
- Hedberg, O. (1964) *Features of afroalpine plant ecology*. Uppsala: Sv. Västgeografiska Sällsk.
- Henn, J.J., Buzzard, V., Enquist, B.J., Halbritter, A.H., Klanderud, K., Maitner, B.S. et al. (2018) Intraspecific trait variation and phenotypic plasticity mediate alpine plant species response to climate change. *Frontiers in Plant Science*, 9, 1548.
- Holmgren, M. & Scheffer, M. (2010) Strong facilitation in mild environments: the stress gradient hypothesis revisited. *Journal of Ecology*, 98, 1269–1275.
- Idbella, M., De Filippis, F., Zotti, M., Sequino, G., Abd-ElGawad, A.M., Fecthali, F. et al. (2022) Specific microbiome signatures under the canopy of Mediterranean shrubs. *Applied Soil Ecology*, 173, 104407.
- Jones, C.G., Lawton, J.H. & Shachak, M. (1994) Organisms as ecosystem engineers. *Oikos*, 69, 373–386.
- Jones, C.G., Lawton, J.H. & Shachak, M. (1997) Positive and negative effects of organisms as physical ecosystem engineers. *Ecology*, 78(7), 1946–1957.
- Körner, C. (2021) *Alpine plant life: functional plant ecology of high mountain ecosystems*. Berlin: Springer Nature.
- Kulichevskaya, I.S., Ivanova, A.A., Detkova, E.N., Rijpstra, W.I.C., Damsté, J.S.S. & Dedysh, S.N. (2017) *Tundrisphaera lichenicola* gen. nov., sp. nov., a psychrotolerant representative of the family Isosphaeraceae from lichen-dominated tundra soils. *International Journal of Systematic and Evolutionary Microbiology*, 67, 3583–3589.
- Labeda, D.P. (2015) *Crossiella*. Bergey's Manual of Systematics of Archaea and Bacteria, 1–7.
- Le Roux, P.C. & McGeoch, M.A. (2010) Interaction intensity and importance along two stress gradients: adding shape to the stress-gradient hypothesis. *Oecologia*, 162, 733–745.
- Liu, Z., Michalet, R., Wang, C., Wang, Y., Chen, J., Cui, H. et al. (2023) Contrasting effects of two phenotypes of an alpine cushion species on understory species drive community assembly. *The Science of the Total Environment*, 859, 160154.
- Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, 97, 199–205.
- Marcenò, C., Gristina, A.S., Pasta, S., Garfi, G., Scuderi, L., Fazan, L. et al. (2022) A multifaceted field sampling approach for the management of extremely narrow endemic vascular plant species. *Ecology and Evolution*, 12, e9477.
- Michalet, R., Brooker, R.W., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Pugnaire, F.I. et al. (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters*, 9, 767–773.
- Molenda, O., Reid, A. & Lortie, C.J. (2012) The alpine cushion plant *Silene acaulis* as foundation species: a bug's-eye view to facilitation and microclimate. *PLoS One*, 7(5), e37223.
- Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D. et al. (2019) The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. *Nucleic Acids Research*, 47, D259–D264.
- Orsenigo, S., Montagnani, C., Fenu, G., Gargano, D., Peruzzi, L., Abeli, T. et al. (2018) Red listing plants under full national responsibility: extinction risk and threats in the vascular flora endemic to Italy. *Biological Conservation*, 224, 213–222.

- Otani, S., Mikaelyan, A., Nobre, T., Hansen, L.H., Koné, N.G.A., Sørensen, S.J. et al. (2014) Identifying the core microbial community in the gut of fungus-growing termites. *Molecular Ecology*, 23, 4631–4644.
- Pignatti, E., Pignatti, S., Nimis, P.L. & Avanzini, A. (1980) La vegetazione ad arbusti spinosi emisferici: contributo alla interpretazione delle fasce di vegetazione delle alte montagne dell'Italia mediterranea. Quaderni C.N.R. AQ/1/79. Roma.
- Pignatti, S., Guarino, R. & La Rosa, M. (2019) *Flora d'Italia*. Bologna, Italy: Edagricole.
- Poli-Marchese, E. & Patti, G. (2000) Carta della vegetazione dell'Etna: note illustrative. Istituto di Biologia ed Ecologia Vegetale, Università degli Studi di Catania, S.E.L.C.A., Firenze.
- Purvis, O., Williamson, B., Bartok, K. & Zoltani, N. (2000) Bioaccumulation of lead by the lichen *Acarospora smaragdula* from smelter emissions. *The New Phytologist*, 147, 591–599.
- Quezel, P. (1967) A propos des xérophytes épineux en coussinet du pourtour Méditerranéen. *Annales de la Faculté Des Sciences de Marseille*, 39, 173–180.
- Reid, A.M., Lamarque, L.J. & Lortie, C.J. (2010) A systematic review of the recent ecological literature on cushion plants: champions of plant facilitation. *Web Ecology*, 10(1), 44–49.
- Ren, C., Zhang, W., Zhong, Z., Han, X., Yang, G., Feng, Y. et al. (2018) Differential responses of soil microbial biomass, diversity, and compositions to altitudinal gradients depend on plant and soil characteristics. *Science of the Total Environment*, 610, 750–758.
- Richter, M. (1984) Vegetationsdynamik auf Stromboli. *Aachener Geographische Arbeiten*, 16, 41–110.
- Rita, A., Bonanomi, G., Allevato, E., Borghetti, M., Cesarano, G., Mogavero, V. et al. (2021) Topography modulates near-ground microclimate in the Mediterranean *Fagus sylvatica* treeline. *Scientific Reports*, 11(1), 1–14.
- Rivas-Martínez, S., Rivas-Saenz, S. & Penas, A. (2002) *Worldwide bioclimatic classification system*. Kerkwerve, The Netherlands: Backhuys Publisher.
- Rodríguez-Echeverría, S., Lozano, Y.M. & Bardgett, R.D. (2016) Influence of soil microbiota in nurse plant systems. *Functional Ecology*, 30(1), 30–40.
- Rosa, L.H., da Silva, T.H., Ogaki, M.B., Pinto, O.H.B., Stech, M., Convey, P. et al. (2020) DNA metabarcoding uncovers fungal diversity in soils of protected and non-protected areas on Deception Island, Antarctica. *Scientific Reports*, 10, 1–9.
- Roy, J., Bonneville, J.M., Saccone, P., Ibanez, S., Albert, C.H., Boleda, M. et al. (2018) Differences in the fungal communities nursed by two genetic groups of the alpine cushion plant, *Silene acaulis*. *Ecology and Evolution*, 8(23), 11568–11581.
- Schöb, C., Armas, C., Guler, M., Prieto, I. & Pugnaire, F.I. (2013) Variability in functional traits mediates plant interactions along stress gradients. *Journal of Ecology*, 101(3), 753–762.
- Sciandrello, S., Minissale, P. & Giusso Del Galdo, G. (2020) Vascular plant species diversity of Mt Etna (Sicily): endemism, insularity and spatial patterns along the altitudinal gradient of the highest active volcano in Europe. *PeerJ*, 8, e9875. Available from: <https://doi.org/10.7717/peerj.9875>
- Selbmann, L., De Hoog, G., Zucconi, L., Isola, D., Ruisi, S., van den Ende, A. et al. (2008) Drought meets acid: three new genera in a dothidealean clade of extremotolerant fungi. *Studies in Mycology*, 61, 1–20.
- Sista Kameshwar, A.K. & Qin, W. (2020) Systematic metadata analysis of brown rot fungi gene expression data reveals the genes involved in Fenton's reaction and wood decay process. *Mycology*, 11, 22–37.
- Soil Survey Staff. (2010) *Keys to soil taxonomy*, Eleventh edition. Portland: USDA (United States Department of Agriculture), NRCS (National Resources Conservation Service).
- Stinca, A., Chirico, G.B., Incerti, G. & Bonanomi, G. (2015) Regime shift by an exotic nitrogen-fixing shrub mediates plant facilitation in primary succession. *PLoS One*, 10, e0123128.
- Stinca, A., Musarella, C.M., Rosati, L., Laface, V.L.A., Licht, W., Fanfarillo, E. et al. (2021) Italian vascular Flora: new findings, updates and exploration of floristic similarities between regions. *Diversity*, 13, 600. Available from: <https://doi.org/10.3390/d13110600>
- Thiers, B. (2022) Index Herbariorum: a global directory of public herbaria. New York botanical Garden's virtual herbarium. Available Online: <http://sweetgum.nybg.org/ih/> (accessed on 22 September 2022)
- Vanegas-León, M.L., Sulzbacher, M.A., Rinaldi, A.C., Roy, M., Selosse, M.-A. & Neves, M.A. (2019) Are Trechisporales ectomycorrhizal or non-mycorrhizal root endophytes? *Mycological Progress*, 18, 1231–1240.
- Venice, F., Colasunno, P., Lombardo, E., Lumini, E., Bianciotto, V., Gargano, M.L. et al. (2023) First morphological and molecular investigation of the mycobiota associated with *Astragalus nebrosensis* in its natural environment (Madonie mountains, Sicily). *Plant Biosystems*, 157, 688–698. Available from: <https://doi.org/10.1080/11263504.2023.2186510>
- Villanueva, P., Vásquez, G., Gil-Durán, C., Oliva, V., Díaz, A., Henríquez, M. et al. (2021) Description of the first four species of the genus *Pseudogymnoascus* from Antarctica. *Frontiers in Microbiology*, 12, 3425.
- Wang, C., Michalet, R., Liu, Z., Jiang, X., Wang, X., Zhang, G. et al. (2020) Disentangling large- and small-scale abiotic and biotic factors shaping soil microbial communities in an alpine cushion plant system. *Frontiers in Microbiology*, 11, 925. Available from: <https://doi.org/10.3389/fmicb.2020.00925>
- Wang, Q., Cheng, C., Agathokleous, E., Liu, Y., Li, X. & Sheng, X. (2021) Enhanced diversity and rock-weathering potential of bacterial communities inhabiting potash trachyte surface beneath mosses and lichens—a case study in Nanjing, China. *Science of the Total Environment*, 785, 147357.
- Wezel, A., Rajot, J.L. & Herbrig, C. (2000) Influence of shrubs on soil characteristics and their function in Sahelian agro-ecosystems in semi-arid Niger. *Journal of Arid Environments*, 44(4), 383–398.
- Zhao, R. & An, L. (2021) Plant size of the alpine cushion *Thylacospermum caespitosum* affects soil amelioration at different elevations. *Plant Ecology*, 222(3), 323–335.
- Zohary, M. (1973) *Geobotanical foundations of the Middle East*. Vols. 1–2. Stuttgart, Germany: G. Fischer.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Species accumulation curve of plant community on Mt Etna based on the number of species (*S*) and cumulative number of successively pooled and randomly ordered samples

Appendix S2. ANOVA results for soil chemical parameters according to elevation and position of *Astragalus* cushions on Mt Etna

Appendix S3. List of plant species, taxonomic family, life form and chorotype recorded at the study site

Appendix S4. Box plot showing data distribution of diversity indices in bacterial (a, c, e) and fungal communities (b, d, f) inside and outside cushions of *Astragalus siculus* at 1650, 1900 and 2210 m a.s.l. on Mt Etna

Appendix S5. Pairwise results of PERMANOVA on data from plant, bacterial and fungal communities showing significant difference in biological communities of how they change inside or outside *Astragalus siculus* cushion plants according to altitude (1650, 1900 and 2210m a.s.l.).

Appendix S6. Pairwise results of PERMANOVA on data from plant, bacterial and fungal communities showing significant difference in biological communities inside and outside *Astragalus siculus* cushions and how they change according to altitude (1650, 1900 and 2210m a.s.l.).

Appendix S7. Difference in relative abundance of phyla from inside to outside the cushions of *Astragalus siculus* for bacteria (a–c) and fungi (d–f) at 1650, 1900 and 2210m a.s.l. on Mt Etna. Black bars refer to standard deviations

Appendix S8. Results from simpler analyses run at species level on bacterial communities with a 70% cut-off on relative abundance at 1650, 1900, and 2210m a.s.l. with relative dissimilarity within the groups

Appendix S9. Results from simpler analyses run at species level on fungal communities with a 70% cut-off on relative abundance at 1650, 1900, and 2210m a.s.l. with relative dissimilarity within the groups.

Appendix S10. Results from simpler analyses run at species level on plant communities with a 70% cut-off on relative abundance at 1650, 1900, and 2210m a.s.l. with relative dissimilarity within the groups

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