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# Constitutive and herbivore-induced emission of Volatile Organic Compounds (VOCs) by *Prunus armeniaca* L. plants infested by *Capnodis tenebrionis* (L.) beetles

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**Key words:** mediterranean flat-headed root-borer, plant-insect interaction, plant volatile analysis, Prunus species, volatile organic compounds

Volatile organic compounds (VOCs) emitted by plants play important roles in biotic-abiotic stress responses (Tholl et al. 2006, Brilli et al. 2019). Capnodis tenebrionis (L.) (Coleoptera: Buprestidae) affects many plant species of Rosaceae, such as apricot, peach and cherry (Bari et al 2019). This pest is subject of plant health provisions of EPPO (European and Mediterranean Plant Protection Organization) as it is increasingly causing the death of the Rosaceae. In this study we analyzed the interaction between the C. tenebrionis (L.) and Prunus armeniaca (L) plants in order to understand the potential role of plant VOCs as infochemical cues in orienteering the insect adults. VOCs analyses were carried by a combination of off-line and on-line methods of VOCs measurements: a branch enclosure system was combined to both gas chromatography-mass spectrometry (GC-MS) analysis in order to identify and quantify VOCs, and to proton-transfer mass spectrometry (PTR-MS) analysis for monitoring time-dependent changes of volatiles (Tholl et al. 2006). The GC-MS analyses allowed the identification of different VOCs in the emission profile of apricot plants. The emission of the C6 and C5 volatiles (aldehydes, alcohols, esters), and several monoterpenes (i.e. linalool, β-myrcene) were induced after the herbivore attack. The PTR-MS measurements showed that emission of several monoterpenes followed a diurnal rhythm, while the release of other terpenes and several VOCs, i.e. C6 compounds, increased as a result of the feeding activity. The obtained profiling of VOCs emissions by apricot provide a pool of chemicals to be assayed using olfactometer and electron-antennography in order to test their influence on the Capnodis behavior and, thus, for developing eco-friendly crop protection strategies (Brilli et al. 2019; Bari et al. 2019).

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# The fungal genus *Trichoderma*: can be useful for the biodegradation of used engine oil?

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Key words: biodegradation, enzymes, hydrocarbons, Trichoderma

Trichoderma is an ascomycete genus that can produce a set of non-specific extracellular enzymes involved in the degradation of different recalcitrant compounds. However, the literature concerning the capacity of Trichoderma of degrading hydrocarbons is still limited. Engine oil is a complex mixture of aliphatic and aromatic hydrocarbons, engine additives and metals; for these reasons, it can be very toxic to living organisms and finding strategies for its sustainable disposal is of great importance. The aim of this work was to investigate the ability of Trichoderma of exploiting used engine oil as the sole carbon source in the prospect of bioremediation of contaminated substrates. Four different strains, isolated in our laboratory and belonging to T. asperellum (F1020), T. atroviride (F12) and T. harzianum (F26, F58), were tested. The fungi were inoculated in a minimum liquid medium in direct contact with used engine oil and after 45 days the samples were analysed by gas chromatography-mass spectrometry (GC-MS). The results reported that all strains (except F1020) significantly changed the oil composition decreasing the aromatic fraction in favour of the aliphatic one. In particular, F26 showed a significant reduction of the BTEX fraction and an increase in short-chain aliphatics C1-C20. Enzymatic tests for laccase and peroxidase have also been carried out, demonstrating that every strain had a different efficacy in the biodegradation of used engine oil and seems to express different mode of action. The genus *Trichoderma* shows characteristics that could contribute in the bioremediation process of substrata polluted by complex mixture of hydrocarbons.

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# Insights into the genome of Antarctic cryptoendolithic black fungi

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Key words: Antarctica, black fungi, comparative genomics, cryptoendolithic communities, extremophiles, stress-adaptation

Black yeasts (namely black fungi or Rock-Inhabiting Fungi, RIF) form a polyphyletic morpho-ecological group in Ascomycota and share a number of characters, such as strong melanisation, thick and even multi-layered cell wall, and exo-polysaccharide (EPS) production, resulting in an extraordinary ability to survive in extreme habitats and tolerate chemical and physical stresses as extreme pH, high and low temperature, desiccation, UV, ionizing radiation and even alpha particles. Studies on the genome evolution of black yeasts, colonizing a diverse array of inhospitable ecological niches, will help to understand the genetic factors that enable their success in extreme niches such as cryptoendolithic communities of Victoria Land, Continental Antarctica. There, the prohibitive conditions are incompatible with an active life on rock surfaces, and microorganisms dwell inside rock porosity to find more protected habitat where and the last chance to survive. Our knowledge on the evolution and adaptation strategies of this peculiar group of fungi remains still scant. We, therefore, generated first draft genomes of seven Antarctic cryptoendolithic strains (*Friedmanniomyces endolithicus, F. simplex, Cryomyces minteri, Rachicladosporium* sp., *R. antarcticum, Hortaea thailandica*, and *Exophiala mesophila*) (Coleine et al. 2017, 2019, 2020) and predicted genes were functionally assigned to the Pfam, MEROPS and CAZy databases. The size of genomes here compared ranged from 23.88 Mb to 46.75 Mb and gene predicted from 8,778 to 18,027, showing evidence for an ancient Whole Genome Duplication in most of cases.

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# Is plant water content a useful tool for predicting the risk of drought-induced mortality?

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Key words: climate changes, drought, tree die-off, water content

Increasing frequency of intense and/or prolonged drought events, as a consequence of ongoing climate changes, has caused widespread mortality of forest trees in different biomes, including the Mediterranean one (Allen et al. 2010). Considering the roles of forests on global carbon and water cycles, tree mortality may have profound consequences on natural ecosystems and crops and are also expected to produce feedbacks on climate change. Therefore, it is very urgent to identify simple and reliable indicators of mortality risk to predict and/or mitigate the negative effects of drought on forests and crops (McDowell et al. 2011). Studies in the past decades have clearly demonstrated that tree die-off under drought is induced by two interrelated physiological mechanisms: plant water transport dysfunction and negative plant carbon balance (Hartmann et al. 2018). However, a reliable indicator of the risk of plant mortality, easy to measure at a large spatial scale and related to the two main physiological mechanisms leading to plant death, is still lacking. In the present study, we measured the relative water content (RWC) of root, stem, and leaf of potted seedlings of *Populus nigra* L. and *Quercus ilex* L. in response to increasing drought. The aim of the study was to check the predictive power of water content for monitoring mortality risk, also considering possible links with remotely sensed estimates of canopy water content. Intriguing correlations between RWC plant organs and different physiological parameters were recorded. Our data support the hypothesis that RWC is a useful tool for predicting the risk of irreversible plant decline and mortality.

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# How vegetation analysis can renew soil bioengineering for slope stabilization

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Key words: ecological indices, monitoring, soil bioengineering, vegetation

Mountain environments play a crucial role in maintaining biodiversity despite becoming more vulnerable to colluvial processes primarily induced by extreme meteorological events. Soil bioengineering stabilizes mountain slopes and limits impacts on ecosystems and is increasingly used worldwide, yet its effectiveness requires better assessment through post-intervention environmental monitoring. However, such studies are only rarely performed even though they are essential to improve future intervention. This research reports soil and vegetation monitoring data of an area in the Italian Alps in which soil bioengineering work was carried out to restore an area hit by landslides. The monitoring involved an analysis of the floristic-vegetational and ecological features of the plant communities of the area of the soil bioengineering intervention, as well as an analysis of the chemical-physical characteristics of the soils where these communities were established. Vegetation analysis was carried out by applying recent ecological indices developed by Giupponi et al. (Giupponi et al. 2015, 2017a, 2017b). The results of the monitoring have highlighted some lines of research and action that should be undertaken by technicians, researchers, and politicians to innovate and to make work aimed at the stabilization of landslides more effective. In particular, it would be extremely useful to study the biotechnical characteristics of herbaceous plants that are still "unknown" in soil bioengineering and to evaluate their possible effects on ecosystems in order to produce seed mixtures that, besides being useful for soil stabilization, can accelerate vegetation dynamics, therefore maximizing the success of such works (Giupponi et al. 2019).

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# Hyphal melanisation in microcolonial fungi: a prerequisite for the penetration into marble?

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Microcolonial fungi (MCF) are a heterogeneous group of organisms known for their ability to tolerate stress on sun- and atmosphere-exposed substrates. The ability of MCF to colonise bare rock surfaces in deserts and on monuments is supported by their constitutive melanogenesis and their ability to penetrate into the depth of solid porous materials. Melanin production has been linked to the mechanisms of hyphal penetration (HP) for phytopathogens MCFs, but few researches focused on its involvement in the penetration of stone materials. The role of melanins in the HP of the MCF Knufia petricola A95 was investigated in standardized lab experiments. We compared the wild-type and three mutants with introduced targeted mutations of polyketide-synthases (melanin production), and/or phytoene dehydrogenase (carotenoid synthesis). Fungi were inoculated on marble powder pellets of different porosity. The HP after 5, 10, 17 and 27 weeks was quantified through the depth and the spread of mycelium into the PAS-stained cross-sections of pellets. These data were collected for (i) melanized toruloid hyphae, with swelling cells typical of MCF growth, and (ii) hyaline, elongated hyphae of smaller diameter. A mycelial growth was observed on and within the pellets for every strain considered, albeit with variabilities in patterns and depths of penetration. Nevertheless, the HP values of the mutant strains did not significantly differ from the wild-type, rejecting the hypothesis of a mandatory involvement of melanin in penetration processes. The possible influence of pellet porosity and environment on HP and mycelium morphological plasticity will be discussed.

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# The role of the arbuscular mycorrhizal symbiosis on tomato resilience to combined stress: from phenomics to functional genomics approaches

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Key words: arbuscular mycorrhiza, drought, RNA-seq, stress resilience, symbiosis, tomato

A predicted outcome of climate change is the reduction in water availability, which can have a severe impact on crop yield (Saadi et al. 2015). Among Mediterranean crops, tomato (Solanum lycopersicum L.) will be one of the most disadvantaged, and innovative solutions, from genotypes selection to growing practices, are required. A sustainable strategy can reside in the exploitation of the beneficial plant microbiota and, in particular, arbuscular mycorrhizal (AM) fungi, that establish a mutualistic symbiosis with roots of most land plants, including tomato. These fungi not only improve plant mineral nutrition, that allows to reduce the use of chemical fertilizers, but also plant resilience to drought (Fracasso et al. 2020). Within the 'TOMRES' European project we are characterizing a panel of selected tomato accessions for their responsiveness to AM symbiosis under combined waternutrient stress. Following a multidisciplinary approach and using a laboratory model system we characterized susceptibility and growth responses to the AM symbiosis of 6 tomato accessions and 2 wild-relative species. Results allowed us to identify responsive and non-responsive genotypes in terms of growth and AM colonization. Notably, S. pennellii, which is known to be drought tolerant, results one of the less responsive genotypes and an RNA-seq analysis of mycorrhizal versus non-mycorrhizal roots suggests that this may be related to some defects in strigolactones metabolism, which are crucial during early stages of the plant-AM fungus interaction. This work will enlarge our knowledge on the effect of the AM symbiosis on plant responses to water and nutrients limiting conditions, possibly highlighting biotechnologically-relevant alleles involved in plant resilience.

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# Mycoremediation of heavy metals in the waters of the Port of Genoa (EU Interreg Project V-A Italy France Maritime 2014 – 2020 "GEREMIA - Waste management for the improvement of port waters")

G. Cecchi, L. Cutroneo, S. Di Piazza, M. Capello, M. Zotti

"Blue Growth" is the European long-term strategy to support a sustainable growth in the marine and maritime sectors. The evaluation of anthropogenic impacts and pressures on ecosystems will be developed on a solid scientific foundation as suggested by modern management approaches (Ecosystem-based Management). Following this approach, pollution risk management must be shared on a cross-border basis. The Interreg Maritime Project "GEREMIA" aims to train and support, with innovative tools and solutions, the management of port waters. The combination of modelling, monitoring and analysis of the procedures of risk management will lead to the preparation of a Decision Support System (DSS) developed for port companies. The strategies of proposal management will be applied in pilot actions on the reality of the Port of Genoa, together with the realization of a method for the mycoremediation and containment of port wastewater, and the exercises of the intervention procedures. In particular, autochthonous marine fungi and selected macrofungi will be employed for the decontamination and ordinary cleaning of the Port of Genoa waters due to the fungal capability to bioaccumulate and bioadsorb pollutants and heavy metals (Cecchi et al. 2019).

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# Assisted phytoremediation of a former mine soil using biochar and iron sulphate: effects on As soil immobilization and accumulation in three Salicaceae species

M. Simiele, M. Lebrun, F. Miard, D. Trupiano, P. Poupart, O. Forestier, G.S. Scippa, S. Bourgerie, D. Morabito

Assisted phytoremediation using amendments is a cost-effective and environmentally friendly approach to control soil pollution (Zhang et al. 2019). However, amendment type, combination and application can influence process effectiveness. In the present study, the effect of two different biochar amendment applications (biochar added on all the soil surface and biochar addition on the soil upper layer) combined with iron sulphate addition was investigated on a former mine soil, highly contaminated by arsenic (Simiele et al 2020). In detail, a mesocosm experiment of assisted phytoremediation was performed in order to evaluate changes in: i) physico-chemical soil properties and ii) growth and metal(loid) uptake of three Salicaceae species (Populus euramericana (Dode) Guinier clone I45/51, Salix purpurea L. and Salix viminalis L.). Results showed that the biochar-iron sulphate combination improved soil characteristics by increasing pH and electrical conductivity and reducing soil pore water metal(loid) concentrations. Between the two biochar application methods, the addition of biochar on all soil surface showed better results. But for such contaminated soil, biochar, in combination with iron sulphate, had no positive effect on plant growth, especially when incorporating on the soil upper layer. Finally, S. purpurea presented high root metal(loid) concentrations associated to the better growth compared to *P. euramericana* and S. viminalis, making it a better candidate for phytostabilization of the studied soil. Further studies are needed to assess: i) the difference between the different ways of applying biochar in field; ii) the combination of biochar and iron sulphate in phytoremediation processes and iii) the mechanisms of interaction involved in plant-bacteria-amendment association in soils contaminated by arsenic.

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# MADS-box genes and development of flower and fruit in *Nymphaea caerulea*: modern traits and old inventions

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Key words: early-diverging angiosperms, evolution, flower development, fruit development, MADS-box genes, water-lily

MADS domain transcription factors are involved in controlling many developmental processes in all the green plants. In angiosperms their roles range from pollen and embryo sac development to root, flower, and fruit development. In some model-species of flowering plants the MADS-box genes responsible for the correct floral organs specification have been extensively studied, while their involvement and characterization in early-diverging Angiosperms is still an open and excited field of study. In this work we isolated and characterized several MADS-box genes in the water-lily Nymphaea caerulea Savigny (an early-diverging angiosperm). Different RNAseq experiments provided transcripts from both floral and fruit tissues. Overall, we were able to identify MADSbox genes related to all traditional function classes. According to the highly complex floral morphology of N. caerulea we characterized a complex expression pattern of the isolated genes in the various floral components. Moreover, with the purpose to discern specific functions of the three AGAMOUS-like genes identified, they were functionally characterized by complementation experiments in Arabidopsis mutants of C and D class. By studying fruit development we discovered that the fleshy multicarpellate fruits form characteristic abscission zones (AZs) which are of a crucial importance for the actual opening of the fruit and the subsequent release of seeds. According to this finding we identified putative key regulators of those specific regions, such for instance a JOINT-LESS-like gene. Among the water-lily reproductive structures it appeared particularly interesting also the presence of the arils around the mature seeds which allow them to float, hence to be dispersed.

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# Small functional foods: phytochemical and nutritional analyses of five Brassicaceae species microgreens

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Key words: anthocyanins, antioxidant activity, Brassicaceae, carotenoids, essential oil, isothiocyanates, polyphenols

Microgreens are early shoots of herbs and vegetables, harvested at the development of their two cotyledon leaves, sometimes at the emergence of their rudimentary first pair of true leaves (Samuoliene et al. 2018, Kamal et al. 2019). Their nutritional profile is high in vitamins, minerals, and antioxidants (Kamal et al. 2019). Indeed, they represent a functional food whose consumption is steadily increasing (Xiao et al. 2019): the species belonging to the Brassicaceae family are among the most used in this consumption form. In the present study, we analysed the essential oil (EO) composition, as well as the content of chlorophyll (Chla, Chlb, Chltot), polyphenols, carotenoids, anthocyanins and ascorbic acid (ASA), and the antioxidant activity of five species of the Brassicaceae family: arugula (Eruca vesicaria (L.) Cav.), broccoli (Brassica oleracea L.), daikon (Raphanus raphanistrum subsp. sativus (L.) Domin), mustard (Brassica juncea (L.) Czern.), and watercress (Nasturtium officinale R.Br.). Isothiocyanates were the most abundant compounds in the EOs of broccoli (4-pentenyl isothiocyanate), mustard (allyl isothiocyanate) and watercress (benzyl isothiocyanate), whilst arugula and daikon exhibited higher contents of monoterpene hydrocarbons (myrcene) and non-terpene hydrocarbons (n-pentacosane), respectively. Arugula exhibited the lowest antioxidant activity, followed by mustard (confirmed by its lowest polyphenol and carotenoid contents, although it had the highest ASA content). Broccoli had the highest polyphenol and anthocyanin contents, with a good antioxidant power, comparable to those of daikon (due to polyphenols and carotenoids) and watercress (due to anthocyanins).

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# Viburnum tinus L.: investigation on its spontaneous from shoot system

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Key words: flower, fruits, laurustinus, leaf, SPME, stem

Viburnum tinus L., commonly named "laurustinus", is an evergreen perennial shrub from Adoxaceae family, indigenous to Africa, Europe, Middle East and is a typical component of the thicket as well as of the Mediterranean under-wood (Alkurdi et al. 2014). Viburnum species have been used in Chinese folk medicine for a long time as sedatives and muscle relaxing (Wang et al. 2008). V. tinus grows spontaneously in Italy mainly along the coast in the Southern regions. It is cultivated for ornamental purposes in parks and gardens due to its showy flowers, berries and fragrance and can be employed as isolated specimen, in group or in mass for hedges. Previous investigation on V. tinus was focused on the isolation and identification of several iridoid glucosides from leaf and branch extracts (Mohamed et al. 2005). The aim of this work was to analyse the aroma profile of V. tinus from different organs, at various developmental stages, using the solid phase micro extraction (SPME). More than 90% of the identified fraction was represented by non-terpenes in both young and adult leaves (94.2% and 94.3%. respectively), as well as in inter fruits of different ages: unripe and ripe (red and black) (93.1%, 90.6% and 90.9%, respectively). This latter class was also the predominant one in crushed fruits (the set of pericarps and seeds) (67.0%); on the contrary, bud and flower emission highlighted a good percentage of oxygenated monoterpenes (80.0% and 75.0%, respectively), while the stem composition was characterized by not-terpenes (34.6%) followed by sesquiterpenes hydrocarbons (29.0%) and a good amount of oxygenated monoterpenes (17.6%).

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# Porphyra dioica J.Brodie et L.M.Irvine; a first look to a mass cultivation of a European species

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Key words: nori cultivation, Porphyra/ Pyropia

*Porphyra/Pyropia* species have an important economic relevance (FAO 2018). This study was focused on *Porphyra dioica* J.Brodie & L.M.Irvine, a European species (Holmes, Brodie 2004) considered suitable for aquaculture (Pereira et al. 2004). The present research aimed to study the influence of different environmental parameters on thalli growth to improve the knowledge on mass cultivation methods. *P. dioica* were collected along the coasts of Galicia. The main target was to test the effects on biomass yield of photoperiod (12L: 12D, 16L: 8D and 8L: 16D) and irradiance (250, 140 and 70 μE  $m^{-2}$  s<sup>-1</sup>). Furthermore, we tested the "free-living" growth conditions on the conchocelis phase, traditionally cultivated on a calcareous substrate. To support the obtained data, the health status of the gametophytes was evaluated by the Fv/Fm index. The highest growth of thalli was obtained at the photoperiod 16:8 and the light intensity of 140 μE  $m^{-2}$  s<sup>-1</sup>. The further increase of light intensity caused a reduction in the growth due to high light stress, as shown by the Fv/Fm index. The cultivation of conchocelis stage in free-living conditions present excellent results.

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# Value-added co-products from biomass of the diatoms Staurosirella pinnata and Phaeodactylum tricornutum

S. Savio

Key words: bioactivity, biorefinery, diatoms biotechnology, drug discovery, lipids

Diatoms are a promising resource for biotechnology (Bozarth et al. 2009), with multiple extractions, and intermediate valorisation protocols able to open new paths in biomedical (Gilbert-López et al. 2017), food and feed (Hildebrand et al. 2012), and bioenergy fields (Wang, Seibert 2017). In this work we have obtained different value-added co-products from a single biomass of two intensively cultivated diatoms strains: the isolated diatom Staurosirella pinnata (Ehrenberg) D.M. Williams & Round, and the model organism Phaeodactylum tricornutum Bohlin. Hydrophilic fractions of crude extracts were characterized for their small metabolites content by Nuclear Magnetic Resonance (NMR), which allowed to identify 52 small metabolites including amino acids, organic acids, sugars and nucleosides. The antiproliferative potential of this fractions was assessed on HaCaT (human keratinocytes) and CHL-1 (human melanoma) cell lines in a 24 h dose-response assay. The results showed that S. pinnata extract had a strong cytotoxic effect on the CHL-1, while no significant cell death was detected on the HaCaT. For *P. tricornutum* extract, we observed a significant cell death only at the highest doses used (10 mg/ml). Lipids were extracted from the residual biomass, resulting from the first extraction step, and their chromatographic profiles evidenced that cis-9-hexadecenoic and eicosapentaenoic acids were the most abundant fatty acids in S. pinnata, while eicosapentaenoic, docosanoic and hexadecanoic acids, were the most abundant in P. tricornutum. Finally, the exhausted biomasses were subjected to anaerobic digestion in order to evaluate their Biochemical Methane Potential (BMP). Our results suggest that exhausted diatom biomass can be a suitable substrate for biomethane production.

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# Microfungal communities in a paddy irrigation system water and effect of fungicides and mechanical management

D. Manzi, G. Cecchi, A.M. Picco

The need of water is crucial for crop production. Nevertheless, water is a potential risk factor. If the water carries undesirable chemicals or microbes, an entire crop may be threatened. Various fungi occur in open irrigation systems (Zappia et al. 2014). Both saprotrophic and pathogenic fungi can be listed and monitored in relation to the agricultural practices taking place along the water flow. In this study a section of the Marocco cable channel, a stretch of irrigation ditch and a rice paddy situated in Vistarino (Lombardy, Italy) were investigated about fungal communities in summer 2018. In the Marocco canal, two mechanical managements have been carried out, one in June and the next in July and August. A low number of colonies forming units (CFUs) and many genera and species were found in the channel on June. On July and August, the number of CFUs increased, while genera and species decreased (from 16 to 8 and from 13 to 6) after the mixing of the bottom. *Aspergillus* species became dominant on August. About the ditch, the CFUs resulted constant and all the main genera were present. The paddy was initially characterized by many CFUs (369), genera (15) and species (19) while, after the fungicide treatment, on July 27th, they significantly decreased (163 CFUs, 9 genera and 13 species). On August 30th fungi started to increase again (565 CFUs, 13 genera and 14 species): this could be due to the efficacy of the fungicide (pre-harvest interval about 30 days). In paddy water fungi reflected this trend: *Fusarium*, *Trichoderma* and *Rhizopus* genera disappeared after chemical treatments.

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# Studies on *Hericium erinaceus* for a better use of its medicinal properties in central nervous system

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Key words: erinacines, Hericium erinaceus, hericenones, strains

Despite the growing popularity of mushroom biomass or extracts as dietary supplements, functional foods and novel prebiotics, the main problem concerns safety, standardization, regulatory aspects, efficacy and information on the bioactive molecules and their mechanisms of action. The aim of the study was to improve knowledge on the effects of *Hericium erinaceus* oral supplementation on cognitive functions during aging in animal models. *H.* erinaceus strains were isolated from specimens collected in Tuscany; based on ITS region, they belong to a strongly supported clade including both European and American strains. Thanks to pure analytical standards neuroactive metabolites erinacine A and hericenones C and D were detected in the following samples: wild and cultivated sporophores; lyophilized mycelium; primordium. Expectedly, erinacine A was recorded from mycelium only; contrarily, hericenones were recorded from sporophores only. Hericenes were also detected in mycelia and sporophores. Cultivated sporophores resulted in significantly higher concentrations than wild ones. *In vivo* tests confirmed and reinforced the hypothesis that recognition memory is made of two distinct components, namely "knowledge" and "remember" in hippocampus and parahippocampus respectively. In order to use a translational approach to transfer all the results obtained in preclinical model to clinical trials on humans, during physiological aging mice were fed with mycelium and sporophore extract mimicing the dose used in humans. Locomotor and cognitive performances were monitored for tuning a frailty index. Two months H. erinaceus oral supplementation were successful in: reverting the cognitive frailty during aging; improving recognition memory; enhancing hippocampal and cerebellar neurogenesis and cell proliferation.

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# Photoprotection in *Selaginella martensii* Spring (Lycopodiophyta) acclimated to different light regimes

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Key words: lycophytes, light regime, photoinhibition, photoprotection, photosynthesis, Selaginella martensii

Selaginella martensii Spring is an extant representative of lycophytes, early divergent vascular plants, and can serve as a model species to study light energy management in ancestral tracheophytes. In fact, this rainforest shade plant is able to acclimate also to high light without significant impairment of its photosynthetic apparatus (Ferroni et al. 2016). Information on the photoprotection mechanisms in this species and, more in general, in this plant group is still incomplete. In this research, the onset of photosystem II (PSII) photoinhibition upon increasing irradiance has been compared in *S. martensii* plants acclimated to extreme shade or intermediate shade or high light regimes, in the hypothesis of a gradient in photoprotection capacity. To this aim we used a PAM-fluorimetry method developed in the last years, which is based on the calculation of the parameter of photochemical quenching measured in the dark (qPd) from light-response curves (Ruban, Murchie 2012). qPd is sensitive to the onset of photoinhibition and is also influenced by PSII antenna uncoupling (Ware et al. 2015). We found that in high-light grown plants the photoinhibition occurring at the highest irradiances was associated with an extensive antenna uncoupling. An opposite scenario characterized extreme shade plants, but photoinhibition at the highest light intensities was surprisingly more strictly controlled than expected for a deep shade plant.

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# What it takes to be a plant growth promoter: characterization of two strains of soil saprotrophic fungi *Minimedusa polyspora* and *Chaetomium globosum*

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**Key words:** applied mycology, *Chaetomium globosum, Minimedusa polyspora*, plant growth promotion, soil saprotrophic fungi

The development of new nature-based solutions for the cultivation of food and medicinal crops is a key strategy to promote environmentally sustainable agriculture. Fungi can promote plants' growth through different mechanisms, such as improvement of nutrition, biostimulation, bioprotection and soil bioremediation from toxic compounds or altered functions (Owen et al. 2015). Therefore, it is crucial to identify and characterize fungal strains with plant growth promotion abilities. This study focused on the characterization of two strains of saprotrophic soil fungi: Minimedusa polyspora (Hotson) Weresub & P.M. LeClair and Chaetomium globosum Kunze. In order to characterize these strains, we evaluated the growth kinetics and the phenotypic profile. Phenotype microarray system allowed, through measurements of respiration and biomass production, to assess strains' ability to use and metabolize a wide range of different substrates. Furthermore, siderophores production is considered an important function related to plants' nutrition improvement and bioprotection. Fungi producing siderophores can promote the dissolution of insoluble minerals and the transfer of iron (Fe), or other cations, into the soil solution as chelates, making them available to plants. Additionally, siderophores play a significant role in the biological control of phytopathogens, since they behave as competitors in Fe uptake (Ahmed, Holmström 2014). Therefore, we also evaluated the production of siderophores of the tested fungal strains by means of the chrome azurol S assay (Milagres et al. 1999). The results of the study showed that the two fungal strains have a high growth rate, which makes them suitable for a biotechnological application. Both of them were able to grow on a wide range of substrates (respectively 50 for C. globosum and 29 for M. polyspora) as resulted from the phenotypic profiles, therefore not requiring particular nutritional conditions. Finally, the chrome azurol S assay showed that both strains can produce siderophores. However, at the same growth-time, siderophores of C. globosum showed a higher chelation activity than those of M. polyspora. In conclusion, the results of this study show that these strains have promising characteristics for plant growth promotion applications, and therefore will be further tested on plants.

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# Homeostatic needs and toxicity of metals: characterization of phytochelatin synthase in the liverwort *Marchantia polymorpha*

E. Bellini, L. Bruno, L. Sanità di Toppi

Key words: Marchantia polymorpha, metals, phytochelatins, phytochelatin synthase

The enzyme phytochelatin synthase (PCS) is a ubiquitous enzyme in land plants, and it is responsible for the synthesis of phytochelatins (PCn) (Grill et al. 1985). Previous studies suggest that the PCS likely plays a pivotal role in the response to metal homeostatic needs and toxicity in all tracheophytes (Gupta et al. 2013), but until now only a few handful investigations have been addressed to the characterization of PCS from bryophytes (Degola et al. 2014, Petraglia et al. 2014). In this study, we focused on the PCS of the model-liverwort *Marchantia polymorpha* L. (MpPCS). In particular, we used a recombinant MpPCS protein, highlighting that the *MpPCS* gene encodes a 530 amino acid polypeptide with a molecular mass of ~57 kDa. In order to evaluate the PCS activation in response to metals, the recombinant protein was used to perform *in vitro* activity assays using Cd, Zn, Cu and Fe. In line with all other known PCSs, HPLC-ESI-MS-MS analyses showed that MpPCS is able to catalyze a transpeptidation reaction and is fully activated by Cd, followed by Zn>Cu and much less by Fe. The PCS role in metal homeostatic needs or toxicity was investigated through *in vivo* experiments in condition of metal excess, physiological concentrations of Fe, Cu and Zn and total starvation of the aforementioned metals. Thus, we were able to demonstrate, via biochemical and molecular analyses, the key role of MpPCS in detoxification of heavy metals and its involvement in homeostatic control of physiological needs of some metal micronutrients.

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# Experimental design to assess integrated phytoremediation approach

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The use of native hyperaccumulator plant species and the related rhizobiota for phytoremediation allows improving the metal uptake by the root system and increasing the phytoextraction of metals (Rosatto et al. 2019). The goal of the study is to standardize an integrated phytoremediation approach to be used in joint bioremediation protocols (plant-rhizobiota). The experimental design and testing in mesocosm of the Nickel-hyperaccumulator species *Alyssoides utriculata* (L.) Medik., and *Noccaea caerulescens* (J. & C. Presl.) F.K. Mey. is presented. The metalliferous soil was sampled in the Ophiolithic Massif of the Voltri Group (Sassello, GE) where total Ni is 1600-2500 mg kg-1 (Marescotti et al. 2019). Selected bacterial and fungal strains have been isolated, screened and consisted of *Trichoderma harzianum* Rifai group 'Serp055' and *Pseudomonas* sp. 'SERP1' (Rosatto et al. 2019). For each plant species, 5 experimental groups were prepared in pot containing approx. 2.5 L of soil: 1) 'Control' (native soil used as is), 2) 'Dry': (dried at 60 °C); 3) 'Fungi' (sterilized at 130 °C and inoculated with *T. harzianum* 'Serp05S'), 4) 'Bacteria' (sterilized at 130 °C and inoculated with *Pseudomonas* sp. 'SERP1'), 5) Mix (sterilized at 130 °C and co-inoculated with *T. harzianum* 'Serp05S' and *Pseudomonas* sp. 'SERP1'). The experimental design consisted of 5 pot groups randomized in 3 rows for both species, to obtain replicates of each group assuring the inferential statistical processing. Before sowing, soils were monitored pre- and post-inoculation of selected bacterial and fungal strains to assess any possible microbial interaction and succession.

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# Do you manage it, or do you want help? Effect of self- and cross-pollination on the seed set of two *Ophrys* species

M. Bazzicalupo, S. Priarone, A. Alberto, C. Turcato, J. Calevo, L. Cornara

**Key words:** cross pollination, seed viability, self-compatibility, self-pollination

Although many plant taxa evolve their floral traits to avoid inbreeding, for other species facultative self-pollination occurs if there are no pollinator visits at all. Selfing is widespread in Orchidaceae but, although it guarantees some reproductive success (Tabalaj et al. 2017), it negatively affects plant fitness and the percentage of embryonated seeds (Jersáková et al. 2006). Therefore, we aimed to compare the effect of self and cross-pollination on the seed quality of two Ophrys species showing different caudicles' flexibility and pollination strategy. The autogamous Ophrys apifera Huds. is known for its flexible columns, which leads to the bending of pollinia on the stigmatic surface; the allogamous O. holosericea (Burm. f.) Greuter, instead, possesses more rigid columns that do not refold on the stigma (Claessens, Kleynen 2002). For each species, we collected 3 self- and 3 cross-pollinated seed pods from different localities. Seed and embryo width/length, percentage of embryonated seeds, and seed viability via FDA test were checked. For both species, cross-pollination produced seeds and embryos significantly bigger. In O. apifera, all seeds from both self- and cross-pollination were embryonated, but embryo viability was significantly higher for outcrossed seeds (53% vs 29%). In O. holosericea outcrossed seeds were 100% embryonated, against 86% of selfed seeds, while seed viability was 51% and 2%, respectively. Our complex of data confirms that *O. holosericea* is self-incompatible, while *O. apifera* is self-compatible although cross-pollination significantly improves seed quality. Our results are useful to obtain a better knowledge of orchid's reproductive biology and for the hand-pollination programs included in LIFEorchids project (LIFE17NAT/IT/000596).

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# Characterization and Antioxidant Activity of Sympatric Orchid Species' Essential Oils from two Ligurian localities

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**Key words:** Anacamptis coriophora, Anacamptis pyramidalis, Ophrys holosericea, Orchis patens, Orchis x fallax, Orchis provincialis, Serapias vomeracea, volatiles

The volatile fraction from fresh inflorescences of terrestrial Mediterranean orchids *Anacamptis coriophora* (L.) R. M. Bateman, Pridgeon & M. W. Chase subsp. *fragrans* (Pollini), *Anacamptis pyramidalis* (L.) R., *Ophrys holosericea* (Burm.) Greuter, *Serapias vomeracea* (Burm. f.) B., collected in Pompeiana (Imperia, Italy), and *Orchis patens* Desf., *Orchis provincialis* Balb. ex Lam. & DC., *Orchis x fallax* (De Not.) Willk. in M.Willkomm & J.M.C.Lange, collected in Lavagna (Genoa, Italy), in May 2016, according to the regional law and with the legal permission of Regional Authorities, was isolated by steam distillation and analyzed by GC/FID and GC/MS. Saturated hydrocarbons were quantified as the major constituents of the volatile fractions (47.87–81.57% of the total essential oil), of which long-chain monounsaturated hydrocarbons accounted from 3.72% to 32.04% of the total essential oils. Aldehydes (from 2.92% to 18.18% of the total essential oils), alcohols (from 0.19% to 13.48%), terpenes (from 0.98% to 2.50%) and acids (0.30% to 16.84%) were also detected. These volatile compounds may represent a particular feature of these plant species, playing a critical role in the interaction with pollinators. DPPH assay of the essential oils was carried out, showing a dose-dependent antioxidant activity.

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# Effect of volcanogenic heavy metals on seed germination of Mt. Etna native plants

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**Key words:** adaptation, seed germination, volcanogenic heavy metals

Volcanic emissions are important sources of metals bearing gases and particles in the atmosphere. However, atmospheric depositions around volcanoes have reached little attentions. A first assessment of atmospheric depositions in the form of rain and particulate was made by Calabrese (Calabrese et al. 2011) on Mount Etna, the most active volcano of Europe featured by a continuous summit activity. Since the prevalent wind direction on Mt. Etna is west to north-west, it was found that deposition of volcanogenic elements from the plume is significantly greater on the downwind, eastern flank of the mountain. Heavy metals carried in the plume may potentially affect plant fitness and seed germination. Main aim of our research is to compare the regeneration by seed of a pool of species at a comparable altitude on the eastern, southern and western flanks of the volcano, differentially affected by plume deposition. The species targeted by the study are: Astragalus siculus Biv., Erysimum etnense Jord., Rumex aetnensis C. Presl, Saponaria sicula Rafin. and Silene sicula (Ucria) Jeanm., and they were selected according to their presence on all investigated stands. Germination tests will be conducted on substrates collected on the three stands, testing seed populations from each site on each substrate until no germination will be recorded for 14 consecutive days. Laboratory germination tests will be coupled with germination tests performed in situ. Finally, the elemental analysis of plant tissue will highlight if there is any differential heavy metal accumulation in the roots, leaves and seeds, and if there are differences among the surveyed sites. Here we present the results of the germination tests. No difference in germination across populations and soils were observed for S. sicula and E. aetnensis. However, significantly less seeds reached the stage of seedling on soils from Galvarina, richer in organic matter. Germination patterns differed significantly between the two populations tested of both A. siculus and R. aetnensis. In both cases the difference could be interpreted as intrinsic to the seed populations (different levels of dormancy and viability) rather than as an effect of the soil. S. sicula did not germinated in any of the condition tested.

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# Plants, people and traditions in the Gran Paradiso National Park (Western Alps, Italy)

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Key words: ethnobotany, ethnomedicine, nature reserve, phytoalimurgia, traditional knowledge

From 2017 to 2019, an ethnobotanical study was carried out in an area characterized by a high naturalistic interest represent by three valleys (Cogne, Valsavarenche, Rhêmes) of the Gran Paradiso National Park (Aosta Valley, Italy). A total of 68 people (mean age 70) were interviewed through extensive dialogues. Informants were requested to indicate the most important plants locally used. Among a total of 199 vascular plants cited, Compositae (25 species), Rosaceae (14), and Lamiaceae (11) were the most represented families. Collected data mainly referred to wild taxa, while cultivated or traded plants were less frequent. A total of 8 categories of use were found, of which the most frequent ones were medicinal (42%) and food (33%). Among medicinal taxa, the most cited were Peucedanum ostruthium (L.) W.D.J. Koch, Malva neglecta Wallr., Juniperus communis L., Arnica montana L., Viola calcarata L., Tussilago farfara L., Cetraria islandica (L.) Ach., and Pinus sppl. Medicinal uses mainly concerning the treatment of respiratory (22%), digestive (19%), and skin (13%) diseases. Among edible species, the most quoted were Urtica dioica L., Taraxacum officinale Weber ex Wiggers, Persicaria bistorta (L.) Samp., and Blitum bonus-henricus (L.) Rchb. Similar to other Italian alpine regions (Pieroni et al. 2009, Cornara et al. 2014, Vitalini et al. 2015) liqueurs made by using Artemisia genipi Weber ex Stechm., Achillea herba-rotta All., Gentiana punctate L., Carum carvi L., and Tanacetum vulgare L. were considered preparations with medicinal properties. The traditional knowledge of plants and their use are still alive in the Aosta Valley, an area also interesting from the linguistic point of view, due to the use of the dialectal language patois. However, many practices are no longer in use, only surviving in elderly people. This rapidly vanishing cultural baggage needs to be studied and documented.

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# Citrus x bergamia Risso & Poiteau: quality assessment and authentication via PCR-based molecular analysis

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Key words: authentication, bergamot, botanicals, DNA, plant dietary supplements, polyphenols

Since ancient time, the use of botanicals played a fundamental role in human health. Nowadays, plant food supplements (capsules, tablets and pills) represent a modern and convenient way to renew traditional practices. These supplements are being perceived by an increasing portion of the population as "natural" and, therefore, "safe", even if their safety strongly depends on correct and conscious consumption. Efficient screening and detection techniques are required to cope with fraudulent practices and unintentional plants swaps in this rapidly growing market. DNA-targeted approaches are officially recognized as a powerful identification tool, especially when the research is focused on processed material where traditional morphology-based methods are not applicable (Böhme et al. 2019). When dealing with intensively processed matrices, a challenging but fundamental step for unequivocal detection is the setup of an optimal DNA extraction procedure, together with the selection of a robust, species-specific molecular marker. In this study, we focus on bergamot (Citrus x bergamia Risso & Poiteau), an Italian product of excellence typical of Calabria area, generally known for its essential oil. Its value drastically increased after the discovery of dyslipidemia-correcting and anti-diabetic effects, determined by a wide pattern of flavonoids with statin-like activity (Janda et al. 2016). We provide here a comparison of different genomic DNA extraction techniques from fruit, spray-dried juice and bergamot-based dietary supplements. Furthermore, we present a preliminary screening for molecular markers specific to bergamot (a natural hybrid), with the aim to develop a multiplex PCR assay, which was proved to be an effective technique for botanical origin authentication of herbal products.

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# Use of two exotic plant extracts against the venom from *Velella velella* (Cnidaria: Hydrozoa)

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**Key words:** *Ananas comosus, Carica papaya*, cnidarians, keratinocytes

Natural compounds from plants, such as phenolics, alkaloids, acids, and proteins can be used to treat evenomation. Traditional herbal medicines are also utilized in marine environments, where venomous stings from cnidarians are one of the main problems for occupational or recreational activities. Cnidarians attract much interest from the pharmaceutical sector: firstly, natural substances produced by these organisms may be potential sources of therapeutics (Killi et al. 2019); on the other hand, the study of protective formulations against their poison represents an interesting commercial objective. Starting from ethnobotanical information about the protective effect of *Carica papaya* L. (Omara et al. 2019) and *Ananas comosus* (L.) Merr. on animal toxins (Gomes et al. 2010), our aim was to investigate the anti-venom properties of their commercial extracts against venom-containing suspensions of discharged nematocyst obtained from the pelagic hydrozoan *Velella velella* L. The cytoprotective properties of the plant extracts were evaluated by *in vitro* cell-culture methods. HaCaT keratinocytes, purchased from DKFZ (Germany) (Boukamp et al. 1988), were co-incubated for 48h with either of the plant extracts and increasing doses of nematocyst venom, and then processed for MTT assay. Data showed that both extracts possess cytoprotective properties and anti-venom potential, with a stronger activity recorded for *C. papaya* with respect to *A. comosus*. These results suggest that the two herbal extracts, especially *C. papaya*, are potentially useful for the formulation of products preventing cnidarian injury to skin.

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# Characterization of polyketide synthases in an ericoid endomycorrhizal fungus using a molecular approach

S. Belmondo, S. Daghino, S. Miyauchi, G. Wu, D. Meloni, C. Aiello, S, Collin, A. Kohler, S. Perotto, E. Martino, C. Jacob

Fungi produce biologically active secondary metabolites, a large proportion of them being polyketides, a group of compounds synthesized by polyketide synthases (PKSs) and characterized by great structural diversity. Several functions have been attributed to fungal polyketides, ranging from ecological and evolutionary adaptation of fungi (Keller 2019) to plant-pathogenic fungi interactions. Potential roles of polyketides have not been investigated so far in mycorrhizal fungi, although a recent comparative genomics and transcriptomics study (Martino et al. 2018) has revealed in Oidiodendron maius Barron, an ericoid endomycorrhizal fungus, the highest number of PKSs encoding genes, some of them being regulated in symbiosis. As O. maius is one of the few genetically tractable mycorrhizal fungi, we started a multidisciplinary project aiming at investigate the potential role of O. maius PKSs including: i) in silico prediction of Biosynthetic Gene Clusters in the genome and analysis of the expression in symbiosis; ii) domain prediction, phylogenetic analysis and comparison of O. maius PKSs with other fungal functionally characterized enzymes; iii) generation of mutants lacking PKSs genes highly regulated in symbiosis, in order to analyse their mycorrhizal phenotype; iv) heterologous expression of these O. maius PKSs in yeast, in order to identify the polyketide produced; v) investigation of the role of O. maius polyketides in biocontrol activities and in vi) response to abiotic stress. Our investigation should provide a picture of O. maius genetic potential in the polyketide biosynthetic pathway and the characterization of some of these compounds and potential role in symbiosis, biocontrol and stress response.

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# Application of forensic botany to veterinary medicine

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Key words: animal poisoning, micromorphological characterization, plant remnants, toxic plants

Plant poisoning of pets and cattle is very common worldwide (Cortinovis et al. 2017). The identification of plant fragments found in biological samples from poisoned animals can provide useful information about the plant involved, allowing veterinarians to adopt suitable therapeutic measures. The aim of this work is to highlight the characteristic features of some toxic plants by Light and Scanning Electron Microscopy, to create an atlas helpful for intoxication diagnosis. Our study was focused on six species, selected on the basis of data reported by Caloni et al. (2013) and by Turnquist et al. (2001), and representative of different contexts and environments. These species included the indoor Christmas plant *Anthurium andraeanum* Lind., the weed *Datura stramonium* L., the outdoor shrub *Nerium oleander* L., the terrace suffrutex *Pelaragonium tomentosum* Jacq., and the grasses *Setaria italica* subsp. *viridis* (L.) Thell. and *S. pumila* (Poir.) Roem. & Schult. Data show that each species has distinguishing features, like glandular and non-glandular trichomes, types of stomata and crystals, enabling the microscopic identification of plant remnants present in the stomach, vomit and faeces of poisoned animals. The micromorphological characterization of remnants can increase our knowledge on the toxic plants typical of a specific geographic area or socio-cultural context. This represents a support tool for veterinarians to improve poisoning diagnosis, and an aid for pet owners to safeguard the health of their animals.

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# Medicinal plants used to treat skin diseases in rural villages of Kavrepalanchowk District, Central Nepal

G. Ambu, L. Cornara

Key words: ethnobotany, medicinal plants, Nepal, skin diseases

The present study examined the traditional uses of medicinal plants by ethnic communities living in some rural villages of the Kavrepalanchowk District (Nepal). We studied 19 medicinal plants belonging to 15 botanical families with a special focus on those used to treat the most common skin problems. People from Kavrepalanchowk, live in rural areas, characterized by poor hygiene and sanitation and the access to the government health system is very difficult (Government of Nepal; http://origin.searo.who.int/nepal/areas/ Strategic\_Priority\_6/en/). Therefore, people highly depend on medicinal plants for primary healthcare and treatment of common illnesses (Ananda et al. 2007, Panthi et al. 2013). Skin diseases represent one of the most diffuse health problems and a serious problem for the population since they cause physical discomforts and can produce discrimination due to ancestral fears and beliefs in rural villages (Kam et al. 2010, Shrestha et al. 2012). Our findings suggest that the most representative botanical families were Asteraceae and Euphorbiaceae (3 species respectively). Only two species were cultivated: Daphne papyracea Wall. ex G.Don (Thymelaceae) and Tagetes patula L. (Asteraceae). The other was wild plants, widespread and easy to find near villages. The herbal remedies were administered through direct application of the plant parts on the injured skin. For medicinal preparations, aerial parts, latex and fruits were mainly used. According to Ethnobotanical indices, Calotropis gigantea (L.) Dryand. (Apocynaceae) showed the highest Fidelity Level value (100%) for dermatological diseases (Saratha et al. 2011). Collected data showed that indigenous people of Kavrepalanchowk District have a rich body of knowledge about flora. Nevertheless, also still poorly explored plant species should be taken into consideration in future phytochemical investigations to study in depth their therapeutic potential.

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# Morphological characterization of the developing ovules of *Ginkgo biloba* and associated molecular pathways involved in the development of the fleshy sarcotesta

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Key words: fleshy sarcotesta, fruits, gymnosperms, Ginkgo Biloba, ovule development

Ginkgo biloba L. is a gymnosperm and is the only representative of the clade Ginkgophyta. Therefore, Ginkgo is morphologically unique in several aspects, including the architecture of its reproductive structures (Douglas et al. 2007). Being a gymnosperm Ginkgo does not produce fruits, and only the seed coat protects the embryo when the seed is mature. The seed coat derives from the ovule integument and differentiates during its development into three different layers: a soft and fleshy outer layer (sarcotesta), a hard and woody middle layer (sclerotesta), and a thin and membranous inner layer (endotesta) (Singh et al. 2008). The outermost layer of the integument becomes fleshy and ripens like a fruit, thus having the functional characteristic of a fleshy fruit, as occurs in many flowering plants. The project aims to understand, from an evolutionary point of view, which are the molecular pathways controlling the transformation of the ovule integument into seed integument, and the mechanisms involved in the differentiation of a single integument into three layers. In order to investigate these processes, we are analyzing transcriptomic data obtained from ovules at different developmental stages, and preliminary results show that the majority of the changes that happen at the transcriptome level, and therefore the main rearrangements in the growing ovule, occur early in the ovule development. Next, we are planning to do in situ hybridization on Ginkgo ovules at different stages, using homologues of selected transcription factors known to be involved in the ovule development and in the differentiation of integuments in model angiosperms, such as MADS-box transcription factors. MADS-box genes are essential for the regulation of reproductive structures development in angiosperms, and similarly, they seem to be involved in ovule and seed coat differentiation also in gymnosperms, enabling them to make their own fruit-like structures (Lovisetto et al. 2011). Together with molecular analysis, we are also studying the morphological features of the developing ovules at different stages to obtain a full vision of the processes.

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# An evolutionary approach for the study of spatial diversity of freshwater macrophytes in Central and Northern Italy

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**Key words:** macrophytes, macroDIVERSITY, phylogenetic diversity, wetlands

Over the past decades, several studies elucidated the effect of the loss of aquatic plant diversity in freshwater ecosystems. Several strategies and approaches for macrophytes diversity conservation have considered taxonomical or functional features, and only in the last years the evolutive dimension of plant communities was also considered (Jordan et al. 2011). Phylogenetic diversity (PD) dimension, reflecting the accumulation of genetic, phenotypic and phenological differences between evolutionary lineages, may have an important role in the biodiversity conservation. Consequently, preserving sites with the greatest amount of phylogenetic variability will, in turn, preserve the greatest variation in organismal features and, in some cases, functions (Winter et al. 2013). From 2020 and 2023, macroDIVERSITY project (PRIN 2017) aims at integrating spectral (SD) and phylogenetic (PD) diversity to map the functional diversity of freshwater macrophyte communities at different spatial scales and trophic gradients. Within the framework of macroDIVERSITY, we will collect data on macrophyte diversity from plots sampled over selected lakes in Central and Northern Italy, based on a robust experimental design. The PD of each plot will be quantified using the Faith's metric. The Net Relatedness Index (NRI) and the Nearest Taxon Index (NTI) will also be used to quantify the degree of the community phylogenetic clustering/overdispersion among species within each plot (Pérez-Harguindeguy et al. 2013). Using the described approach, we aim to 1) delineate the evolutionary relationships within the wetland communities; 2) obtain PD metrics of each macrophyte community/ecosystem; 3) achieve a feedback on wetlands genetic sustainability of Central and Northern Italy.

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# A possible endophytic symbiont of *Androsace brevis* (Hegetschw.) Cesati (Primulaceae)

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Androsace brevis (Hegetschw.) Cesati is a narrow endemic plant living on ridges in a restricted area of Southern Alps in Lombardy and Switzerland, preferring acid soils with low nitrogen content. This species is proposed as a model to study biotic interactions in mountain ecosystems. During A. brevis genome sequencing, carried out in a previous work, a significant amount of prokaryotic DNA not compatible with an environmental contamination was detected. The aim of the present work is to identify and characterize this microorganism. The bacterial genome was de novo assembled and identified as belonging to the Beijerinkiaceae family, Rhizobiales order. The Beijerinckiaceae family includes bacteria living in the phyllosphere, often methylotrophs or methanotrophs sharing N-fixation capability and promoting plant growth. To evaluate the diffusion of the bacterium, specific PCR primers were designed and tested on *A. brevis* specimen belonging to eight different natural populations: the presence of the identified bacteria was confirmed in all samples tested. We tried to isolate the bacterium from plant tissues using different growth media and conditions; unfortunately, all attempts were unsuccessful, so far. The bacterial genome was investigated to identify predicted functional domains, confirming a likely symbiotic relationship with the plant and the ability of the bacteria to metabolize methane as source of carbon and energy. We plan to test more growth media and conditions and to extend the analysis to the microbiota of A. brevis and other alpine species living the same area, to investigate the fascinating network of plant-microbe relationships existing in such challenging environments.

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# Co-cultivation of *Tisochrysis lutea* and *Nannochloropsis oculata*: preliminary tests on cultivation medium and starter inoculum variations

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Key words: co-cultivation, microalgae, Nannochloropsis oculate, Tisochrysis lutea

Nowadays, the great biodiversity of microalgae and their different biochemical composition make them attractive as commercial sources of a wide range of biomolecules (vitamins, aminoacids, PUFA, pigments, etc.) (Borowitzka 2013, Matos 2017). Many studies aim at lowering production costs, optimizing culture systems. In this context, some studies have focused on co-cultivations of different organisms, a strategy based on community ecology approaches (Zhu 2015). In this regard, the aim of our research is to test the co-cultivation of *Tisochrysis lutea* EL M.Bendif & I.Probert 2013 (Haptophyta) and Nannochloropsis oculata (Droop 1955) Hibberd 1981 (Ochrophyta) to obtain compounds, such as pigments and lipids. In the preliminary phase of experimentation, we used f/2 medium and inoculum of 0.6x10<sup>6</sup> cells ml<sup>-1</sup> for *T. lutea* and 5x10<sup>6</sup> cells ml<sup>-1</sup> for *N. oculata*. Both microalgae did not benefit from co-cultivation, especially T. lutea. However, an increase in the concentration of chlorophylls and carotenoids was shown in both strains. At the end of experiment, lipids were analysed by GC-MS: there was no increase in the amount of lipids in co-cultivated cells as compared to the mono-cultivated ones. An improvement of algal co-cultivation still needs further research. To this purpose, we are preliminarily testing co-cultivation in SWES medium with addiction of vitamins and inoculum of  $1x10^6$  cells ml<sup>-1</sup> for both strains. Co-cultivation in SWES stimulated growth of N. oculata. T. lutea grew better in mono-cultivation than co-cultivation, but monocultivated *T. lutea* grew better in SWES than f/2. Further researches will permit to set up optimal culture conditions for the co-cultivation of the two microalgal strains.

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# Chitin oligomers extraction from fungal biomass and their use as promoters of arbuscular mycorrhizal symbiosis

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Chito-oligosaccharides (COs) are chitin and chitosan derivative molecules. Several studies confirm the presence of COs (Myc-COs) in germinated spore exudates from arbuscular mycorrhizal (AM) fungi, the widespread root symbionts that improve plant nutrition and stress resistance. When perceived by host plants, Myc-COs activate a symbiotic signalling, which includes the triggering of repeated oscillations in nuclear Ca2+ concentration and regulates the expression of AM-related genes and root colonization by the symbiotic fungi (Genre et al. 2013, Volpe et al. 2019). These discoveries point to possible applications of Myc-COs to enhance AM establishment in crops. Currently, commercial COs are obtained from fishing waste processing industries. This presents some drawbacks due to seasonal availability and environmental pollution. The aim of this work is to isolate, characterize and to test the biological activity and the AM development of Myc-COs obtained from the biomasses of three fast-growing fungal species, as a low-cost alternative to commercially available molecules. NMR analyses were used to characterize products from each fungus. Purified Myc-COs were applied to Medicago truncatula Gaertn. root cultures to confirm their ability to trigger nuclear Ca2+ spiking in epidermal cells. Lastly, the effectiveness of the best-fungal vs crustacean Myc-COs treatment was verified in M. truncatula potted system. Our results indicate that the purification protocol efficiently isolates bio-active Myc-COs from both fungi. Production scale-up, the choice of cheaper substrates for fungal culture and an optimization of the extraction protocol are expected to reduce costs, making the use of Myc-COs in agricultural context an achievable goal in the near future.

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### Biotechnological strategies to improve salt stress resistance in *Brassica napus* L.

M. Rossi, G. Platamone, M.M. Del Gallo, C. Forni

Key words: Brassica napus, Plant Growth Promoting Bacteria, salt stress, seed priming

Salt stress is an enormous worldwide problem for agriculture. The presence of salts in soils, due to wrong agricultural practices together with climatic changes, leads to a severe plant growth reduction. Salt stress induces, at cellular level, a severe osmotic and oxidative stress, which severity depends on salt concentration. To counteract this stress plants can rely on morphological and physiological responses, depending on their genotype and the intensity of the stress. Entailing these responses, with pre-sowing treatments on seeds, is a good strategy to overcome salt stress. *Brassica napus* L., a salt sensitive plant (glycophytes), is an important plant for biodiesel, food, fodders and secondary metabolites productions. Salt stress leads to a severe biomass reduction in *B. napus* plantations, causing a severe economical loss. As mentioned before, pre-sowing treatments on seeds can help plants to overcome salt stress. In our work we are testing the effect of seed priming and inoculation with Plant Growth Promoting Bacteria (PGPB) as a strategy to entail *B. napus* salt tolerance. Both strategies gave us good results (enhanced roots and shoots length, reduction of membrane damaging, higher amount of phenols and proline to overcome respectively oxidative and osmotic stress and enhanced enzymatic antioxidant activity), entailing *B. napus* growth in highly salinized soils.

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### Toward understanding differences in lateral root formation from *Arabidopsis* root in primary and secondary structure

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Key words: pericycle, vascular cambium, xylem pole pericycle

The control of root system architecture is a fundamental part of plant development, enabling plants to grow in changing environmental conditions. Lateral root (LR) formation and growth is one of the most important factors that controls root system architecture. In the primary root, the competent pericycle cells are necessary to renew mitotic activity to become founder cells of the lateral root (Möller et al. 2017). The root in the secondary structure is also able to produce new lateral roots in sectors in which the primary tissues (including the pericycle) are released. Recently, Chiatante et al. (2018) have suggested that only some stem cells in the vascular cambium of the roots are competent to initiate secondary lateral roots, and their competence may be inherited directly from those competent pericyclic cells that were in front of the xylem. This competence could be correlated to the ability of the cells of the pericycle to perceive specific signals and, in turn, to activate/suppress specific genes as well as the transcription factors necessary to trigger the initiation events of the lateral root. To verify this hypothesis, three *Arabidopsis thaliana* (L.) Heynh. xylem pole pericycle cell marker lines (J0121, GATA23 and RALF34) were used to identify by confocal analysis the competent cells able to initiate LR formation in the primary and secondary root (Pariotz et al. 2008, De Rybel et al. 2010, Murphy et al. 2016). Successively, to evaluate similarities and differences between primary and secondary lateral root formation processes, specific molecular factors and mechanisms will be deciphered by using tissue/cell "omic" analysis.

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### Fungal education and outreach aims

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Key words: environmental education, fungi, mycology, outreach

The Laboratory of mycology works on different areas of mycology and disseminates the results through intense outreach activity. The latter is aimed at teaching people about the world of fungi, their relationships with other living organisms, their role in nature, and their importance for humanity (Timmis et al. 2019). To achieve this purpose the Natural History Museum and the Botanic Garden of Genoa have been designed two activities for schools. These practical activities are devoted to the students from 10 to 19 years old and they are called "Funghi, virus, batteri, questi sconosciuti!" and "Piante e funghi contro l'inquinamento". The first run in cooperation with the Laboratory of Microbiology, the last with the Applied Botany Laboratory of University of Genova. The University of Genoa organizes events where it is possible to speak about fungi. In the last few years, the laboratory of mycology participated in several events like "Festival della scienza", "Festival del mare", and "UniverCity". These activities were carried out by a group of students and/or educational guides with the support of the mycology laboratory staff. While the concept of a plant or an animal is quite easy to understand also for a child, more complex is teaching what a mushroom is to people in general. Since the kingdom of Fungi is an important part of communities and everyday life, it is very important to disseminate information on mycology both for adults and children.

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### Latitudinal and elevation gradient differently drives species understory coexistence in beech forests of Apennine chain (Italy)

A. Bricca, F.M. Tardella, M. Cutini, A. Catorci

Key words: beech forests, climatic filtering, community-weighted-mean, functional diversity, plant trait

The research aim was to understand whether and to what extent the latitudinal range affects the functional structure of the herbaceous layer of calcareous beech forests in Apennines, managed as high-forest. We selected 163 plots (20 x 20 m), ranging from central to southern Italy, using a random stratified sampling design. We chose plant traits related to clonal strategies, storage organ, leaf phenology and reproductive strategies. We correlated the effect sizes of traits' CWM, and functional diversity (calculated as FDis and Rao's Q), with the main axes of variation in species composition, linked to the latitudinal range and elevation. Despite the weak variation in species composition along geographic range, functional diversity of storage organs and vegetative spread resulted negative correlated with the latitudinal range. Functional diversity of pollen/spore dispersal system was negatively related to elevation. Our study suggested that different environmental conditions filter different strategies across large-spatial scales (Grime et al. 2006). Increasing of winter cold stress towards central Italy seems act as a climatic filter on clonal strategies, suggesting that persistence of species is guaranteed by the presence of particular clonal strategies (increasing rhizome and bulbs, decreasing runner and root with adventitious bud) and storage organs (secondary storage root) rather than leaf or reproductive strategies. Contrary, the persistence in higher elevation was ensured by the presence of reproductive strategies shared by the species (i.e., decreasing of wind pollination and increase of insect pollination). Finally, we provide an evidence that FDis and Rao's Q are equivalent metrics in detecting the assembly rules.

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### How fine-scale abiotic and biotic variations affect taxonomic diversity in abandoned sub-Mediterranean grasslands

A. Ferrara, A. Bricca, A. Catorci

**Key words:** abandoned grasslands, abiotic features, biotic features, taxonomic diversity

Abandonment of semi-natural grasslands throughout Europe is causing the invasion of coarse tall grasses with competitive stress-tolerant strategies, leading to a decrease in biodiversity (Louault et al. 2002). Our questions were i) Does the variation in micro- and mesic-scale features affect dominant species and taxonomic diversity of plant communities? ii) Does the increase of dominant species affect negatively taxonomic diversity? The study area was "Montagna di Torricchio" Natural Reserve (central Apennines). In abandoned grasslands dominated by Bromus erectus Huds., Sesleria nitida Ten. and Brachypodium rupestre (Host) Roem. & Schult., we randomly selected 53 plots (5x5 m) recording plant species cover, topographic and soil variables. We ran RDAs to investigate the effect of abiotic variables on dominant species and the effect of both biotic and abiotic variables on species richness, evenness and exponential of Shannon index. We assessed the single contribution and combination of abiotic variables, dominant species, and B. rupestre cover on taxonomic diversity through Variation Partitioning. S. nitida was found dominating less dry grasslands whereas B. erectus the xeric grasslands. Furthermore, B. erects and B. rupestre were found on sites which had deeper soils, suggesting the soil as the main driver for the spreading of B. rupestre. Both abiotic features exerted a significant effect on plant community. Flat landforms and deep soils tend to enhance taxonomic diversity. Finally, we found a negative effect of B. erectus and S. nitida on plant community, in agreement with the prediction of competitive exclusion exerted by dominant species (Grime 2001).

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### Comparative seed germination patterns of alpine plants in the context of climate change

F. Porro, S. Rosback, G. Rossi, A. Mondoni

In alpine habitats seeds are considered the main vehicle for plant migration (Parolo et al. 2008), establishment (Walck et al. 2011), and persistence (Schwienbacher et al. 2010). Regeneration from seeds is under strong environmental control, suggesting that climate change will inevitably affect recruitment success. Here we evaluated the role of germination patterns as a driver of species response to climate warming. To this end, seeds of 76 species, collected from five long-term monitoring sites (project GLORIA, http://www.gloria.ac.at/) depending on whether their population a) increased, b) decreased or c) remained stable, were exposed to six incubation temperatures (T) in the laboratory (5°, 10°, 15°, 20°, 25°, 30° C), with and without a cold stratification (CS) period. Germination data were analyzed with GLMs. We hypothesized that 1) species increasing in nature germinate better at warmer temperatures, 2) have a wider temperature range for germination, and 3) are less dormant than stable and decreasing species. Increasing species showed the highest final germination percentage at the widest range of temperatures. Contrary to our hypothesis, these species germinated better at cold T (5°C), after CS. Species from the other groups showed lower seed germination, with a high percentage at high T (20, 25° C), after CS. Our results indicate that species able to germinate at low temperatures are favored in a present and a future warmer climate, by eliciting seedling emergence right after snowmelt, when water is available and drought hazards are low. On the contrary, germination synchronized with higher temperatures may promote seedling emergence in seasons becoming less suitable with climate warming.

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### Intraspecific variability is the main driver of functional changes in lichen communities

P. Hurtado, M. Prieto, G. Aragón, F. de Bello, I. Martínez

Key words: climate, functional trait variation, intraspecific variability, latitudinal gradient, lichen, species turnover

Traditional approaches in trait-based community ecology typically expect that trait filtering across broad environmental gradients is largely due to the replacement of species, rather than intraspecific trait adjustments. Unfortunately, few studies quantify the relative importance of species turnover versus intraspecific variability mediating the response of communities different from vascular plants. To fill this gap, we studied the functional changes in epiphytic lichen communities within 23 beech forests across large latitudinal and environmental gradients in Europe to quantify the relative contribution of species turnover and intraspecific variability and the role of climate controlling community-level trait changes. Our results showed that intraspecific trait variability explained most of the functional changes in lichen communities in response to the latitudinal gradient. Further, such functional changes were determined by the covariation between intraspecific trait variability and species turnover, which varied in sign depending on the trait considered. Finally, different climatic predictors explained functional variation due to both intraspecific trait variability and species turnover. We propose that lichen communities cope with contrasting climatic conditions by adjusting the functional trait values of the most abundant species within the communities rather than by the replacement of the species. Consequently, intraspecific variability should be explicitly incorporated to better understand the effect of environmental changes on lichen communities, even over large environmental variations. Our results challenge the universality of the hypothesis that species turnover chiefly drives functional trait changes across large environmental gradients and call for a wider test of such important assumptions in trait-ecology in different organism types and ecosystems.

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### High-mountain plant-pollinators interactions: the case of the narrow endemic alpine plant *Androsace brevis* (Hegetschw.) Ces. (Primulaceae)

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Climate change is a key threat to biodiversity. In Europe, plant species that most suffer its effects are those restricted to mountains environments. Some of these are characterized by a very early flowering, that occurs just after snowmelt. An emblematic example of that is Androsace brevis (Hegetschw.) Ces. (Primulaceae), a narrow endemic species that lives only on peaks and ridges of siliceous mountains above 2000 m a.s.l. in the western Alps (Lombardy) and adjacent Switzerland (Ticino) within an area of 800 km<sup>2</sup> Climate warming represents a serious menace to this species, since the upward shift of its range is almost impossible. Moreover, an anticipation of its already very early flowering could cause mismatches with pollinators. We investigated the reproductive biology of A. brevis and the role of arthropods as pollinators through the identification of flower-visiting arthropods and quali-quantitative palynological analysis, to discover the main pollinators of this species and reconstruct the pollination network that characterize these high-altitude ecosystems in early season. We sampled flower-visiting arthropods belonging to 30 families and to 8 orders. We observed pollen belonging to 23 plant families and A. brevis pollen was observed only on Hymenoptera and Diptera that were also the most abundant taxa. In conclusion we demonstrated the important role of Diptera Anthomyiidae and Hymenoptera Apoidea as pollinators of A. brevis and we assessed the taxa spectrum active on its flower. These results are important to explain the pollination biology of A. brevis and can provide the basis to develop conservation plans applicable also to other threatened alpine species.

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## Videorecordings as an innovative method to investigate the behaviour of arthropods on high altitude plants. The case study of *Androsace brevis* (Hegetschw.) Cesati (Primulaceae)

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Androsace brevis (Hegetschw.) Ces. is a narrow endemic plant that grows on siliceous peaks and ridges above 2000 m a.s.l. in the western Alps of Lombardy and neighbouring Switzerland. This species is representative of mountain species threatened by the ongoing climate warming. In particular, A. brevis is characterized by a very early flowering, occurring immediately after snowmelt (May-June) and lasting few weeks. Therefore, a key risk for this plant could be represented by climate change-driven mismatches between its flowering and pollinator presence and activity. Recent studies have found that A. brevis needs pollinators to ensure its reproduction; a pollinator network has also been identified through the capture of the arthropods visiting the flowers. To evaluate the occurrence and ethology of A. brevis flower-visiting arthropods in-field video recording sessions were conducted. Environmental parameters were also monitored to investigate their influence on recorded arthropods activity. We recorded 165 videos for a total of about 42 hours and analysed them with a behavioural observation software (BORIS). Our results allowed the identification of taxa involved in A. brevis pollination, but also revealed the existence of a great variability in the activity and behaviour among flower-visiting arthropods, suggesting that this plant can represent an essential resource for high-altitude arthropod community. This innovative approach, less invasive than human direct observation and sampling, is expected to represent a powerful instrument to finely define the relationship between plants and associated fauna in this particularly vulnerable environment, providing crucial knowledge for their conservation.

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### Subalpine vegetation changes triggered by mining and livestock husbandry close to a recently excavated medieval settlement (Piani di Sasso, Lombardy Alps)

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Key words: archeology, forest, human impact, mining, paleoecology, pollen, pasture

We present a new paleoecological record from a peat bog close to a medieval settlement in the subalpine Valle Brembana, Lombardy Alps. The present-day vegetation structure of the area is the result of interaction between climate change and human activity since the Late Neolithic/Bronze Age (Furlanetto et al. 2018). A recent archaeological excavation at the "Piani di Sasso" (1690 m a.s.l.) unearthed a settlement dating back to the Early Medieval Period. The site was possibly an iron ore processing site. Iron mines are historically attested in the valley at least from the High Middle Ages (Cucini Tizzoni 1994, Riceputi 2004), as supported by dated charcoal burner sites located close to the settlements. Interestingly, such a medieval village was (still is) lying close to a peat bog archive which was investigated to provide information on cultural interference on ecosystems. The paleoecological analysis, supported by radiocarbon dating, is aimed at reconstructing the vegetation history all along the period of strong human impact including prior pristine forests. Pollen spectra point to dense spruce forests during the II millennium b.C., followed by an opening during the Iron Age testified by increasing abundance of pollen from shrubs (Alnus viridis (Chaix) DC. and Corylus). After the III sec b.C. the record of Juglans starts, slightly anticipating Castanea. From the IV/V century AD a peak in charcoal deposition mirrors the exploitation of local woody resources to power the furnaces. Charcoal production and livestock husbandry triggered the demise of pristine subalpine forests into pastures. In the Late Middle Ages charcoal deposition decreases, while pasture proxies persist with high values.

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### The effect of exotic plant species on phylogenetic diversity of common reeds communities

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Key words: common reeds, exotic plant, phylogenetic diversity

Macrophytes have a pivotal role as point of interaction between many of the organisms, and the water and sediment chemistry in a freshwater system. Despite their low floristic diversity, reeds beds can support a wide range of wildlife, and provide a variety of functions for the wetland ecosystems. Recent studies show the existence of a threshold effect of alien plant cover or density on plant communities dominated by *Phragmites australis* (Cav.) Trin. Ex Steud., whereby more increased the alien plant impact is, more reduced may be the native plant species diversity (Houlahan, Findlay 2004, Thomaz, Michelan 2011). However, many of these studies were based on taxonomical or traits inform ation (van Kleunen et a. 2010), whereas no investigations have considered the phylogenetic dimension so far (Lishawa et al. 2019). This study aims to investigate the incidence of alien species on the phylogenetic diversity of wetland vegetation dominated by P. australis. To do this, we investigated the floristic composition of a total of 98 natural reeds beds from Tuscany, Umbria, and Lazio, analysing separately lentic and lotic communities. We constructed a phylogenetic tree of the plant species and compared the differences in taxonomic/functional/phylogenetic diversity, evaluating the role of the incidence of the alien species. The results showed that the relative abundance of exotic plant species is positively related to the taxonomic diversity of the common reeds communities and doesn't affect the phylogenetic diversity. Probably, the human impact promoted the entrance of the exotic species already represented at evolutionary level. Also, the presence of exotic species influenced functional diversity, especially it promoted the adaptive strategies ruderals of the communities.

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### Habitat 3170\* - Mediterranean temporary ponds (Directive 92/43/EEC): distribution and characterization in Liguria

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Key words: amphibious vegetation, microhabitat, phytosociology, temporary ponds, conservation priority

According to the Council Directive 92/43/EEC, several "micro-habitats" are fundamental for biodiversity preservation. Specifically, Mediterranean temporary ponds (code 3170\*), a priority habitat, host many rarefying species, mainly amphibian therophytes (Isoëto-Nanojuncetea). We have investigated habitat 3170\* in Liguria (North-West Italy), where it is formally listed in several Special Areas of Conservation, but its present distribution is largely unknown, due to their phytosociological interpretation which is controversial because of its affinity with other amphibian habitats. We analyzed a floristic richness map (obtained from 600 occurrences of 90 species related to the habitat) through three Mediterranean climatic indices and photointerpretation, identifying 90 potentially suitable sites. Where in the selected sites an amphibian vegetation occurred, phytosociological surveys and transects were performed. According to our results, a relevant vegetation (belonging to the order Nanocyperetalia flavescentis Klika) occurs only in small patches in three sites along the Magra and Vara valleys (East Liguria). The only best-preserved site occurs within a swamp system (Magnocaricion elatae Koch) in the Magra's alluvial plain, while the others occur on river muddy banks, showing many catenal contacts with both aquatic and amphibian habitats (e.g. 3140, 3260, 3270). These results confirm the exceptional rarity of Mediterranean temporary ponds in Liguria. However, according to the phytosociological interpretation proposed by several authors (Evans 2006, Bagella et al. 2007, Mariotti 2008) the surveyed sites should more correctly be attributed to the habitat 3130 (Oligotrophic to mesotrophic standing waters with vegetation of Littorelletea uniflorae and/or of the Isoëto-Nanojuncetea), suggesting that the habitat 3170\* is probably absent in Liguria.

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# Temporal changes in the floristic composition, ecology and structure on high mountain grasslands: preliminary observations in the Matese LTER Site (LTER\_EU\_IT\_022)

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Key words: Ellenberg indicator, global warming, life forms, vegetation monitoring

Several ecosystems are threatened by the effects of climate change and high mountains ecosystems are among the most sensitive ones (Thuiller et al. 2005, Gottfried et al. 2012). The present study aims to analyze floristic and functional changes in high mountain ecosystems occurred during the last decade in a massif of Central Apennines (Matese massif, Italy) and its relation with global change. We analyzed floristic data collected according with GLORIA protocol (GLobal Observation Research Initiative in Alpine environments) (Pauli et al. 2004) in two summits in the years 2007 (T1) and 2015 (T2). In the same sites we have registered daily soil temperatures. We explored floristic data by Principal Component Analysis (PCA), followed by similarity percentage procedure (SIMPER) for identifying species related with different time steps, summits and aspects (N, E, S, W). We examined vegetation structure and ecology using the life form categories of Raunkiaer (Raunkiær 1934) and the Ellenberg indicators (Ellenberg 1974). Specifically we tested the presence of significant differences in the two time steps using Mann Whitney test. Our results underline significant variations in floristic composition and an increase of some life forms as *Hemicryptophytes Caespitose* mainly because the expansion of *Festuca laevigata* Gaudin. The analysis of the Ellenberg bioindicator values shows an increase in T (Temperature value), specifically of montane (T4) and euri-mediterranean (T7) species. The monitoring data of Apennine vegetation could be part of a wider analysis aimed at improving our knowledge on ecological changes in alpine vegetation.

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### Different plant communities hosting *Ionopsidium savianum* (Caruel) Arcang. in Tuscany: taxonomic and functional diversity

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Key words: community ecology, CSR strategies, functional traits

This research analyses two different plant communities hosting *lonopsidium savianum* (Caruel) Arcang., a species of European concern (Gigante et al. 2014). Despite these two communities present common traits guaranteeing the presence of *I. savianum* (e.g. no presence of trees, high solar irradiation), they occur in areas ecologically very different, particularly concerning the substratum type: limestone (Monte Calvi) and serpentine (Monte Pelato). The communities have been analyzed focusing on taxonomic  $\alpha$ - and  $\beta$ -diversity, functional diversity and ecological strategies sensu Grime (Grime, Pierce 2012). Taxonomic β-diversity has been studied using the SDR simplex model by Podani et al. (2013), while the functional traits measurement has been based on the main functional traits according to literature: Leaf area (LA), Leaf dry matter content (LDMC) and Specific leaf area (SLA). The adaptive strategies have been determined following Pierce et al. (2016). Our results highlight significant differences in both taxonomic α-diversity, showing a greater number of species on Monte Calvi, and β-diversity, resulting in a higher value through the comparison of the two sites. Measurements of functional traits in Monte Calvi community presented a higher level of SLA and conversely a lower LDMC, with regard to the measurements in Monte Pelato community. The analysis of ecological strategies showed greater importance of the S strategy (stress-tolerant species), emerging more evident in Monte Pelato community, whereas R strategy (ruderal species) resulted more important in Monte Calvi one. Accordingly, we can conclude that the two communities differ significantly from each other, confirming that the substrate type leads to relevant taxonomic and ecological and functional differences.

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### How biyearly mowing and *Brachypodium rupestre's* features affects plant community

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Key words: Brachypodium rupestre (Host) Roem. & Schult., Mediterranean grassland, mowing, plant trait

Land-use cessation in sub-Mediterranean grasslands causes the spread of dominant unpalatable tall grasses, causing a drop in the biodiversity and affecting ecosystem functioning (Louault et al. 2002). Mowing is known to increase plant diversity after invasion (Bonanomi et al. 2006) but the mechanisms acting at fine scale are poorly investigated. This research was aimed to understand the effect of biyearly mowing events and variation of Brachypodium rupestre's plant height and phytomass on the fine scale functional composition of a grassland invaded by B. rupestre. Since 2010 in Central Apennines, we fenced a grassland invaded by B. rupestre, and we divided it in two blocks, half of each mowed biyearly and half unmown. In 2017 we selected twenty 20 x 20 cm plots per block, collecting data on species occurrences, B. rupestre height, and phytomass. We selected traits related to life history, plant architecture, seed mass, vegetative propagation, storage organ, leaf anatomy and phenology. We performed linear mixed-effects models to investigate the effect of B. rupestre plant height and phytomass and recurrent mowing on functional composition of the community. Overall, recurrent mowing and changes of B. rupestre features affects the plant community at micro-scale leading to changes in their functional composition. Recurrent mowing fosters the spread of avoidance strategy species (e.g. annual life span) and tolerance strategies species as well (e.g. clonal strategy). Increasing of B. rupestre height promotes temporal phenological shift of the coexistence species and foster the presence of taller species, more able to compete for light acquisition (Lepš 2014).

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# Ecological features of supraglacial plant communities along the Alps and the role of Debris-covered glaciers as refugia for cold-stenotherm species

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Key words: climate change, cold-adapted species, Debris-Covered Glaciers, refugia

The number of Debris-Covered Glaciers (DCG) is increasing due to the climate change (Citterio et al. 2007); such landforms are assuming a key biological role: the debris cover has a strong impact on the evolution of glaciers, reducing the rate of ice ablation, but can also allow the colonization of glaciers by many organisms. Indeed, despite of the instability of the substrate and the severe microclimatic conditions, the supraglacial debris could provide a refuge for cold-adapted plant species (Tampucci et al. 2017). Comparing surveys of plant species performed throughout different sites in the Alps, within the Alpine altitudinal belt, it is possible to describe the ecology and the composition of supraglacial plant communities. For this work these sites have been chosen: Peirabroc glacier (Maritime Alps, Cuneo), Amola glacier (Adamello group, Trento), Sorapiss glaciers (Dolomites, Belluno). Data were collected performing vegetation surveys in 12 plots for each glacier on the glacier debris, each consisting of a 5 x 5 metres square area. Soil samples were taken in each plot to describe the main soil features influencing plant growth. The mean number of species for glacier is six; among these taxa, the genus Saxifraga is represented on every glacier. The biological spectrum consists of two components: Hemicryptophytes and Chamaephytes, despite the diverse specific and chorotype composition. Species are cold-adapted and require medium humidity values, low humus content and low-medium values of nutrients. The specificity of ecological requirements of these species and the different specific composition of DCGs, with respect to nearby iceless landforms, confirm the important role of DCG as refugia during warm periods (Fickert et al. 2007).

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### Quantifying habitat suitability for narrow endemic alpine plant species in the Dolomites (Italy) under predicted climate change

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Key words: alpine narrow endemic plants; climate change; Dolomites, species distribution models

The Dolomites include nine protected areas and have been declared UNESCO World Heritage. They host unique endemic alpine plants that are threatened by climate change having a narrow geographical and altitudinal distribution range because of their eco-evolutionary history. To assess the potential habitat loss under predicted climate change, we forecasted the change in habitat suitability for four narrow endemics (Campanula morettiana Rchb., Rhizobotrya alpina Tausch, Saxifraga facchinii W.D.J. Koch. and Sempervivum dolomiticum Facchini). We used 320 occurrences from the available floristic records. First, to test the importance of topography in determining species distribution we built models using climate, topography and the combination of both variables. Then we forecasted future habitat suitability under four different climate change scenarios (rcp 4.5 and 8.5 for 2060 and 2080) based on five different General Circulation Models from the CHELSA climate database at 1 km resolution. No significant difference was found among climate and topography models, suggesting a strong relationship between climate and topography. The habitat loss will increase over time and it will be more severe in the worst scenario. S. facchinii will experience the highest range reduction varying from 44% to 79%, according to year and scenario. *C. morettiana* and *S. dolomiticum* will experience the lowest decrease ranging from 20% and 23% to 62% and 66%, respectively. The suitable habitats will persist in higher elevations reducing connectivity among populations. This study assesses the vulnerability against climate change of these rare and unique species with the aim to foster conservation actions in the Dolomites.

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# Sustainable management practices in Mediterranean agroforestry systems using the Adaptive Multi-Paddock model (project Life Regenerate - Revitalizing multifunctional Mediterranean agrosilvopastoral systems using dynamic and profitable operational practices - LIFE16 ENV/ES/000276)

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Key words: Agenda 2030, grazing, pastoral value, sustainability

Sustainable management of agroforestry systems allows achieving several Sustainable Development Goals (2030 Agenda) as these systems are critical for biodiversity conservation and provisioning of ecosystem services, such as genuine food, cultural aspects, and carbon sequestration (Lee et al. 2019, Niamir-Fuller, Huber-Sannwald 2020). In this view, LIFE Regenerate aims to enhance the biodiversity and ecosystem services in Mediterranean agroforestry systems through the application of the Adaptive Multi-Paddock (AMP) management model. This involves rotational grazing with high instantaneous stocking rates and resting periods long enough to allow an optimal plant regrowth between two successive grazing. The experimental design includes two permanent grasslands, one at low altitudes (PG\_la), one at high altitude (PG\_ha), and a wooded grassland (WG), with 4-8 paddocks each and the same number of control areas. The point quadrat method along permanent transects was applied for vegetation surveys (Bagella et al. 2013). Basing on the specific contribution of presence, according to the experimental design, we compared plant assemblage composition using NMDS, PERMANOVA, and SIMPER analysis and biodiversity indices using ANOVA. To evaluate the primary ecosystem services provided by these systems, e.g., forage provision, we calculated the Pastoral Value (Seddaiu et al. 2018). At baseline time, we found differences between the three trials in plant assemblage composition (Pperm=0.001 for all comparisons), richness (PG la=63, WG=55, PG ha=49) and Pastoral Value (WG=52.5, PG la=40.1, PG ha=26.9). In the following years since the start of the trial, the rotational grazing produced diversified effects on vegetation and Pastoral Value. The results are discussed to evaluate possible options for sustainable management tailored for different environmental conditions.

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### Functional phytosociology of woody Italian vegetation

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Key words: climatic belt, plant functional traits, plant growth form, plant strategies

Vegetation has been traditionally studied through phytosociology, with a taxonomical community approach. Nonetheless, over the last few decades, has been broadly acknowledged that community functions largely depend on the characteristics of each species, in particular on resources acquisition and use strategy. Such approach relies on plant functional types and traits which have been widely applied in recent plant community research, but a link between phytosociology and functional ecology still has to be investigated. The aim of this study was to characterize main woody communities of the Italian vegetation, from Mediterranean to Alpine climatic belts, in order to identify differences, common features and trends in ecological functionality. We considered about 200 species, measuring leaf traits and collecting data about canopy height and seed mass. We performed Principal Components Analysis and ANOVA with post-hoc tests. We also calculated Grime's CSR plant strategies with StrateFy method (Pierce et al. 2017). The species were displayed along two principal axes of variation, one representing the Leaf Economics Spectrum and the other representing the Size Spectrum. Overall, species of extreme habitats were significantly different from species of temperate environments; trees and shrubs in particular showed smaller, thicker and more conservative leaves with a tendency toward the stress-tolerant (S) strategy, while species of intermediate climatic belts had bigger, but thinner, acquisitive leaves, being in general more competitive (C). This study allowed a preliminary functional analysis of Italian woody vegetation, highlighting convergence in ecological strategies of *syntaxa* of extreme climatic belts.

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### Palynology to investigate environmental transformations on a long-term perspective in the Po Plain: the case study of the Terramara S. Rosa di Poviglio

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Key words: climate change, interdisciplinary, long-term perspective, pollen, Po Plain

Palynology is a key science to investigate long-term environmental transformations. The study of the "Terramara Santa Rosa di Poviglio" (1550-1170 BC) was funded by the project SUCCESSO-TERRA (PRIN-20158KBLNB -Human societies, climate-environment changes and resource exploitation/sustainability in the Po Plain in the mid-Holocene: the Terramare culture; PI M. Cremaschi, A. Zerboni - Università degli Studi di Milano; https://www.successoterra.net; Cremaschi et al. 2018). The geoarchaeological-palynological approach helps to show how climate (dry phase c. 3.6 ka cal. BP) and human actions (over-exploitation of the resources) act in synergy to shape woods and fields during the development and collapse of the Terramara culture. Palynological spectra (pollen, fern and moss spores, non-pollen palynomorphs-NPPs) together with microcharcoal analyses add information to archaeological and geoarchaeological data and radiocarbon dating. Three off-site cores were collected at different distances north from the site in 2018 (Cremaschi et al. 2018 unpublished) with the aim to verify the presence of a Po River palaeo-riverbed and to collect data on vegetation and landscape through about 300 pollen samples. Pollen analyses show that land use has been already well-attested during and after the Bronze Age, and included open areas with synanthropic plants, cereal cultivation and pasture fields. Woods largely consisted of broadleaved oaks and other deciduous trees, while wet environments were quite spread with phases of expansion and reduction. The data from the off-site analyses, compared with the on-site studies (Cremaschi et al. 2016), allow reconstructing regional vegetation changes (Mercuri et al. 2012) and specific adaptive behavior of the Terramare people as a result of the synergy between climate oscillations and human impact over a wide, regional, area.

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### The pastoral landscapes of arenaceous substrata in the Monti Sibillini National Park (Central Italy)

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Key words: bioindication, cluster, grasslands, phytosociology, plant community ecology

The "Monti Sibillini National Park", representing and important hotspot for biodiversity in Central Italy, is distinguished by calcareous substrates (90% of the surface) in its south-eastern foothills, and arenaceous sediments outcrops (5% of the National park territory). The vegetation of this hilly landscape is mainly composed by forest ecosystems, arable fields and grasslands/meadows. In the last decades the abandonment of mountain farms and the cessation of traditional land-use led to a huge expansion of woods and scrublands (Mazzoleni et al. 2004, Falcucci et al. 2007); contrariwise fields and, most of all, grasslands suffered a marked reduction of their extension. Because of such considerations it is mandatory a deeper understanding of the ecology and taxonomic composition of the grassland/meadow communities. During the survey campaign (May-June 2008), we performed 55 phytosociological relevées, using the Braun Blanquet's method. These surveys were then processed to cluster analysis that highlighted the presence of five different clusters reflecting five different plant communities. For each community we defined the phytosociological placement, ecology and structural features, the taxonomic relevance for the flora of the National Park and the status of conservation. Two clusters were represented by mown meadows dominated by Arrhenatherum elatius (L.) P.Beauv. ex J.Presl & C.Presl, and Cynosurus cristatus L. respectively. The other two clusters were characterized by an acidophilus, semi-mesophilous grassland dominated by Nardus stricta L. and a xeric grassland, mostly abandoned, characterized by Bromopsis erecta (Huds.) Fourr. and Achillea tomentosa L. Finally, the last was dominated by Calluna vulgaris, a locally strongly threatened species that in this territory have one of the southernmost populations of the Italian peninsula.

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# Cross-taxon analysis of vascular plants and ants in a Mediterranean agroforestry system: first results from the project "Innovative beef cattle Grazing systems for the Restoration of Abandoned Lands in the Alpine and Mediterranean mountains" (iGRAL-AGER2)

G. Piga, M. Verdinelli, M.C. Caria, M. Marrosu, P.P. Roggero, S. Bagella

Key words: biodiversity, grassland management, relate analysis, traits

The characterization of complex biodiversity patterns across ecosystems requires substantial effort, expertise, and financial resources, so choosing surrogates as a shortcut to predict biodiversity changes is essential. Surrogate identification is primarily based on cross-taxon congruence analysis (Wolters et al. 2006). In this study, we analysed vascular plant and ant assemblage composition and richness in a Mediterranean mountains agroforestry system (Macomer, NU) in order to evaluate and compare their response to different management types. Indeed, ants are successfully used as indicators of land management (Andersen, Majer 2004, Bagella 2014, Mauda et al. 2018) while vascular plants are a focal biodiversity surrogate (Reyes-López et al. 2003). The experimental design includes four different management types along a gradient of land use intensification: total abandonment, improved grazing, ordinary grazing, restoration areas and different vegetation cover (grasslands, clearings, open woodlands, close woodlands). Three replicates were considered for each sampling units. Vegetation surveys were performed using the point quadrat method along permanent transects. Ants were sampled close to the vegetation transects, using a set of four pitfall traps that were buried in the ground and arranged in a square grid of approximately 10 x 10 m. As a whole, 221 plant species and 21 ant species were found. The cross-taxon analysis shows a significant congruence between vascular plant and ant assemblages (Relate analysis,  $\rho$ =0.634, p=0.01). Moreover, ant richness was significantly correlated with Legumes cover (R=0.697, p=0.05) and also with Poaceae cover (R=0.796, p=0.01). The results are discussed considering different implications related to plant and ant traits in relationship to land management.

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### Plant invasions in the Mediterranean basin

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Key words: alien species, "ideal invader", Mediterranean ecoregion

One of the major characteristics of human alteration of the environment is the erosion of biogeographical barriers caused by human mediated dispersal of alien species outside their native range. Many alien species naturalise and become invasive causing ecological, economic and social damage. However, to date we still have only very gross estimates of invasions in Europe and in particular in the Mediterranean Basin, which is considered a biodiversity hotspot in terms of species, endemisms and ecological functions (Chytrý et al. 2008). We aim to bridge this gap by analysing one of the largest available datasets of vascular plant species occurrences in Europe, obtained from the European Vegetation Archive (EVA). We collated 381741 georeferenced vegetation plots falling within the Mediterranean ecoregion of Europe and identified 522 alien species, which vary in their degree of spread and local success in the area. We quantified invasion trajectories of these species (van Kleunen et al. 2015) and found that other Mediterranean regions of the world have been the main donors of successful alien species to the Mediterranean basin, suggesting that environmental filtering is among the major drivers of invasion in this area. These results are confirmed by further analyses pinpointing the general characteristics of the "ideal invader" in the Mediterranean basin (Richardson, Pyšek 2006). Our results quantify for the first time the extent of alien plant naturalizations in the Mediterranean basin, and illustrate the urgent need to control, manage and understand the spread of alien species in this vulnerable area.

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### Exploring dynamics of high elevation vegetation under changing climate

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High elevation ecosystems are among the most threatened by climate change and will experience remarkable species range shifts, loss, and community composition change, with great concern for their rich and peculiar biota (Gobiet et al. 2015, Pepin et al. 2015). In this scenario, improving the prediction on the biotic effects of climate change is crucial (Marini et al. 2011, Nascimbene, Spitale 2017). According to a multitaxon-approach including vascular plants, bryophytes, and lichens, this study aims at exploring the dynamics of the photoautotrophic biota of high-elevation environments that are triggered by climate change, as well as by local factors. Our sampling design is based on elevational transects that have been established both in the Alps (on calcareous and siliceous substrates) and in the central Apennines (Majella massif). The methodological framework integrates species functional traits with community diversity analyses mainly focused on species richness and beta diversity. Our preliminary results suggest an evident effect of the elevational gradient in determining the pattern of lichen communities (vascular plants and bryophytes are still under identification). Moreover, in the Majella massif we have found some arctic-alpine species that are restricted to the highest part of the elevational gradient and that are at their southernmost European distributional range. These species have a typical disjoint distributional pattern with the nearest localities of occurrence located in the Alps.

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### Beach litter ecological effects along the Central Adriatic coast of Italy

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Key words: beach litter, coastal habitats, dune ecosystems, plastic waste

The presence of macro litter on sandy beaches and dunes represents a widespread and serious environmental problem, which threatens coastal dunes integrity across the world. The study provides new insights on the accumulation of beach waste in the coastal environment and its impact in coastal dune habitats of European Concern (EC) in southern Abruzzo and Molise. We collected data in April and May 2018, using square plots (2x2m) randomly distributed in the EC habitats occurring along the dune vegetation zonation (Stanisci et al. 2014, Sperandii et al. 2017). The waste was classified according to the type of material and origin (OSPAR Commission 2010) and the entity of its accumulation in each EC coastal habitat was evaluated. The collected data were processed by applying the Mann Whitney test (Past Statistic software). Plastic and polystyrene are the most abundant materials and the litter associated with fishing and boating activities represent half of the waste sampled. The EC habitats most affected by beach litter are the foredunes and the river mouths, however the most volatile waste, like polystyrene, also reaches Mediterranean scrub, juniper and back dune pine forests (de Francesco et al. 2019). Recent studies have shown that, once incorporated into sediments, plastic bags and compostable polymers can reduce the success of sexual recruitment and the growth of new settlements of native dune species seedlings (Menicagli et al. 2019). In perspective, the following step of our research will focus on exploring the ecological impact of polystyrene on the germination ecology of native and alien species.

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### Alpine shrub encroachment in the central Appenines: current patterns and temporal trends

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Key words: alpine vegetation, landscaped dynamics, shrubs encroachment

Since the end of the 2nd World War, European mountain areas have been increasingly abandoned, resulting in grazing declines and often in dwarf-shrub encroachment within alpine and sub-alpine belts (Komac et al. 2013). Apennine mountains are characterized by relatively low altitudes and affected by Mediterranean summer drought that makes vegetation particularly vulnerable to climate change (Lionello et al. 2006). According to IPCC reports, drier summers are expected to occur in the Apennine area with 30-40% less precipitation by 2100. In this work, we aimed to describe current patterns and encroachment trends of shrubs in the context of the ongoing changes in climate and land use. The study focuses on Central Apennines (Reatini, Duchessa and Ernici mountains) where we mapped the shrubs cover on current aerial photos and on historical photos (1954). We then related current shrub cover and encroachment trends to factors related to geomorphology, primary productivity (NDVI), climate (including snow cover), and grazing pressure. Preliminary results show the current spatial distribution of shrubs is mainly negatively related to primary productivity and grazing pressure. There has been significant shrub encroachment in the area, mainly by *Juniperus communis* L. Grazing abandonment, next to climate change, is an important driver of this trend. These results are in line with the ecological characteristics of Juniperus as a frost and drought tolerant species and light demanding invader (Thomas et al. 2007) who encroaches mainly in unfertile habitats (such as rocky outcrops and screes). Against this background, future results will help shed light on the role of soil characteristics for potentially mitigating or exacerbating these patterns.

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### Pollinator diversity and efficiency in Apple orchards in Emilia-Romagna

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Key words: apple, biodiversity, organic management, palynological analysis, pollinator

Apple (*Malus domestica* Borkh) is one of the most important and widespread fruit crops pollinated by insects globally. The majority of cultivars are self-incompatible and rely on insects (mainly bees) for cross pollination (Garratt et al. 2014, Földesi et al. 2015). The present study investigates the pollinating role of taxonomically distinct insects visiting apple flowers and describe the guilds of pollinators in four apple orchards managed with different agricultural systems (IPM and Organic). Pollinators have been monitored to assess their diversity and visitation frequency, and the pollen they carried has been identified to estimate their fidelity to *M. domestica*. A "pollinator importance" index (PI) was calculated as an estimation of the efficiency of each pollinating taxon. Pollinator taxonomic richness was similar between the orchards, while diversity indices highlighted some differences. In general, *Apis mellifera* L. was the most abundant bee, followed by bumblebees (*Bombus* spp.) and mining bees (*Andrena*), while the mason bees (*Osmia*) were the less one represented. These genera showed also the higher PI values (Ballantyne et al. 2017). Alongside, the palynological analysis allowed to estimate the "beeplant" diversity within the orchards and their surroundings: the highest number of flowering species was found on the organic orchard system, supporting the positive correlation between organic management and biodiversity (Porcel et al. 2018).

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### Patterns of vegetation succession at broad geographical scales: the time is ripe for comparative studies and meta-analyses

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**Key words:** disturbances, Europe, restoration, succession

Current successional theory lacks an integration across broad geographical scales. Most successional research addresses site or process specific questions, but extrapolation of the findings to broader spatial and temporal scales is rare. Comparative studies of either many seres or one sere over a broad geographical scale are really scarce. In this project we would like to analyse vegetation succession across broad geographical scales with a focus on the latitudinal gradient in Europe. For this purpose, we are preparing the European Database of Successional Series (EDaSS), using our own and literary data and, moreover, data that will be provided by researchers all over Europe that kindly will contribute to our Database. We aim to compile successional data of changes in vegetation following disturbance (mining, abandonment from agriculture, forest clearing, fire, land-slides, dunes, emerged bottoms, glacial retreat). We will test hypotheses especially concerning the probability a succession reaches a target stage, rate of succession, type of trajectory, trends in species number and the number of target and invasive species, all in relationships to latitude, climate, and biomes. Besides contributions to the theory of ecological succession, we expect implications to ecological restoration, and nature conservancy across the continent.

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### Long-term changes in *Carex curvula* community composition; preliminary results on Orobian Alps

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Key words: climate change, Landolt indices, phytosociological relevés

Climate change is supposed to heavily affect climate-limited communities such as Alpine grasslands. The occurrence of phytosociological relevés performed during the last century provides the opportunity of monitoring floristic and structural changes occurred in the last decades. The present study aims to investigate how Carex curvula All. communities changed during a time span of c. 30 years. We compared 22 published and unpublished phytosociological relevés collected during the 1990s (Andreis 1996, Andreis et al. 1996, Caccianiga et al. 2000) with new data collected during 2019 in the same sites, identified through the original station data. Relevés were selected considering late successional communities of the alpine belt, mainly *C. curvula*-dominated grasslands. All the relevés were analysed with multivariate analysis; the ecological profile of each relevé was assessed with Landolt's ecological indices (Landolt et al. 2010). Cluster analysis identified seven main clusters, from dry to snowbed communities. Landolt indices showed an overall increase temperature value in the new relevés. The humidity values showed a slight reduction, while the nutrient values increased. Humus values showed a consistent increase, especially concerning dry communities. Light values decreased in almost all the observed communities. This suggests an upward shift of shadow-requiring species as a consequence of temperature increase. In general, we observed a deterioration of pure C. curvula communities, the decrease of cold-demanding species and the appearance of species linked to human impact, like *Nardus stricta* L. The following step of our research is to extend the analysis to all the other relevés taken during the 1990s in the whole area.

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### Nectar trait variability in Buglossoides purpurocaerulea (L.) I.M.Johnst.

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Key words: Buglossoides purpurocaerulea, nectar composition, nectar variability, pollination ecology

Floral nectar is the main reward offered to pollinators. The behaviour and efficacy of the pollinators themselves are affected by nectar traits, but the latter may vary within the same plant and even the same flower, depending on intrinsic and extrinsic factors (Schaeffer et al. 2016, Palmer-Young et al. 2019). In this study, we investigated whether the nectar of Buglossoides purpurocaerulae (L.) I.M.Johnst. (Boraginaceae, Lithospermae) changed through functionally different floral stages and after insect visits. B. purpurocaerulea is a self-incompatible plant relying on pollinators for its sexual reproduction. Flowers show incomplete proterogyny and no hercogamy, thus self-pollination could potentially occur. The studied population is situated close to Bologna, in the protected area SIC-ZPS IT4050001. Nectar samples were collected from different floral stages and from both bagged and unbagged flowers. HPLC analyses were performed to investigate amino acid and sugar composition. We found that nectar volume and sugar abundance are affected by floral phenology as well as by insect visitation. When visits are prevented, the older flowers contain more nectar but lower sugar concentration. These results suggest nectar sugar reabsorption in unvisited flowers. At the female stage, nectar shows a lower sugar content but as concentrated as in the other phenological stages. In contrast, the amino acid profile does not change during the flower lifespan, being relatively rich already in the bud. In particular, the high concentration of  $\beta$ -alanine, which is likely to affect pollinator preference because of its involvement in functional flight muscles, possibly explains why the female stage is already attractive to insects (Bogo et al. 2019).

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# Experimental evidence of the consumption of the invasive alien duckweed *Lemna minuta* by herbivorous larvae of *Cataclysta lemnata*. Are we facing a potential biocontroller?

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Key words: aquatic weed management, biocontrol, biological invasion, free-floating macrophyte

Alien plant invasion is a serious threat for biodiversity conservation. One worrying example in Europe is the American duckweed *Lemna minuta* Kunth (Araceae: Lemnoidae) that, since its arrival in the 1940s, has become widespread throughout many countries (Ceschin et al. 2018). Controlling its growth has become a pressing issue and, since chemical and physical methods for its removal are as risky for the aquatic environment health (Rzymski et al. 2013) as ineffective (Ceschin et al. 2016), we focused our study on the biocontrol of this species, searching for a native insect that could consume *L. minuta*. We chose the moth *Cataclysta lemnata* L. (Lepidoptera: Crambidae, Acentropinae) as a potential biocontroller, since it is an herbivore with a diet including preferentially duckweeds, such as the native *L. minor*. We tested the effectiveness of larvae at three different instars in consuming *L. minuta* under laboratory conditions. Larval preference for the alien *L. minuta* and the native *L. minor* was also investigated. In this study it is shown for the first time that larvae of *C. lemnata* can feed on *L. minuta* (Mariani et al. 2020). Moreover, they feed effectively, and seemingly without preference, on both the native and the alien species, contrary to the Enemy Release Hypothesis (Keane, Crawley 2002), which assumes that native consumers are better adapted to consume native species than alien ones. *C. lemnata* late-instar larvae were more efficient in *Lemna* consumption. This study suggests that *C. lemnata* could be a potential candidate to be adopted as biocontrol agent of *L. minuta*.

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## Effect of grassland's mosaic structure and dynamism on the habitat suitability for the *Alectoris graeca* singer male in a central Apennine pastoral landscape

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Key words: Brachypodium genuense (DC.) Roem. & Schult., grazing cessation, habitat suitability, rock partridge, singer male

Over the last several decades, the Mediterranean region has been subjected to mountain abandonment and farming cessation (leading to vegetation changes and grassland coenological features) contextually to the strong decline of the rock partridge (Alectoris graeca Meisner) (Bracchetti et al. 2012). To investigate the interplay of the presence/absence of singer males and the composition and dynamism of the vegetation mosaics, the sampling design consisted in two phases, aimed to define the suitable sites of spring territorial singer males (Angelici et al. 2001), and to characterize the vegetation mosaic of such sites. We defined the presence/absence of spring territorial singing males in seven sites (1,250-2,400 m a.s.l.), by the census in 74 playback stations, distributed along 15 transects. We characterized the topography and the vegetation mosaic of such sites according to the collected topographic and vegetation cover data in 59 randomly selected plots (100 m × 100 m) along the transects. Our results emphasized the marked overlap between the general environmental condition proved to define the habitat suitability for A. graeca and the characteristics of the site chosen by the singer male for starting the reproductive activities. Tall grass-dominated communities and thick-turf grasslands exert their negative effect decreasing the habitat suitability for singer male starting from very low cover values (Catorci et al. 2011). Therefore, the singer male's suitable habitat will be dramatically restricted to the steepest aspects of the south-facing slopes, where topographic and soil conditions do not allow the spread of closed vegetation and the invasive/dominant tall grasses expansion.

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### Diversity of succulent species in the native flora of Sicily

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Key words: convergent evolution, ecology, Mediterranean

The succulent syndrome represents a significant example of convergent evolution across several families of vascular plants determined by ecological conditions with a remarkable water stress (Griffiths, Males 2017). The occurrence of succulent tissues is not the only character used to define succulence, since it is often associated with a peculiar kind of photosynthesis, called Crassulacean Acid Metabolism (CAM). In any case the definition is not univocal and varies according to the authors. The greater biodiversity of these species is found in central-southern America, South Africa and Madagascar. As regards concerns the Mediterranean area and Sicily in particular, the succulent species represent just a minor fraction of the whole native flora, chiefly (Fici et al. 1997) belonging to few families, such as Crassulaceae, Amaranthaceae and Apocynaceae (Fici et al. 1997, Guglielmone et al. 2009). Main aim of our study is to realize an inventory of succulent species in Sicily, provided with some relevant data related to their morphological diversification and ecological requirements.

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### A morphological and phytosociological comparison between Jacobaea persoonii e Jacobaea incana in the Ligurian Alps

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Key words: J. incana, J. persoonia, morphological, narrow-range, phytosociological, variability

In a center of endemism, like SW-Alps, narrow-range endemics often occur in parapatry with their wide-range congeners. The relationships between such closely related species might help to clarify speciation processes and pattern of endemism. Nevertheless, detailed morphological and ecological comparative analyses often lack. We compared Jacobaea persoonii (De Not.) Pelser. (endemic to Ligurian Alps) and J. incana (L.) Veldkamp, (widespread in W-Alps), using morphological and phytosociological features of populations occurring along their contact-zone. We defined the distribution of J. persoonii through bibliography, herbaria and field observations. Phytosociological surveys were performed on five populations for each species. We collected vegetative and reproductive morphometric data, considering also individuals showing an intermediate appearance (putative hybrids), occurring in one site where the species are sympatric. Morphometric data were analyzed through Principal Component Analysis. Our surveys confirmed that distributional ranges of the two species are not overlapped. Despite its narrow range, *J. persoonii* occurs in a wider variety of vegetations (from the heaths of Loiseleurio-Vaccinion to the calcifuge vegetation of Asplenietea trichomanis) than I. incana, which mainly occurs in acidophilic prairies (Caricetea curvulae). According to morphometric analysis, the two species are distinct, and the putative hybrids are mainly included in the variability of *J. incana*, that showed the highest morphological variability. Overall, despite the lack of geographic isolation, the species are differentiated from both morphological and vegetational perspectives. Surprisingly, the narrow-range J. persoonii colonizes a wider range of vegetation types, suggesting that the differences in ecological requirements are probably important to maintain species boundaries.

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### Investigations on ecology and distribution of *Senecio inaequidens* DC. in Tuscany

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Biological invasions are today an important component of human-induced global environmental change (Vitousek et al. 1997, Parmesan et al. 2003) and a serious threat to native species and biodiversity (Weber 2003, DAISIE 2009, Bellard et al. 2016). They lead to the alteration of ecosystem function (Vilà et al. 2011), economic losses and relative control costs (Scalera 2010, Pyšek et al. 2012, Vilà et al. 2015). Senecio inaequidens DC. is an alien species with one of the fastest expansion rate. In Tuscany, before the results of the present study, literature data were scarce and it was considered (Galasso et al. 2019) as naturalized. Our aim is to define the current distribution of S. inaequidens in Tuscany, investigating the biological characteristics and verifying its occurrence in sites historically recorded and in other ones with ecological conditions suitable for it. In particular, we followed a large portion of Tuscany's railway lines, which proved to be effective dispersion corridors (Lachmuth et al. 2010), due to the bio-ecological characteristics of S. inaequidens. Indeed, it exploits the air currents, due to passing transport, for the dispersion of seeds (Blanchet et al. 2015). Data collected in the field were georeferenced in GIS environment, allowing to produce a map of the current distribution of this species and an estimation of its current population size. Its distribution in Tuscany can be generally considered to date not alarming, since the plant spreads mainly in secondary anthropogenic environments, particularly along railways and roadsides. However, the current extent of *S. inaequidens* in Tuscany is considerably larger, compared to past bibliographical reports, and thanks to our survey its status changed from naturalized to invasive. Moreover, four sites are reported as hosting large populations with risk of further spreading, for which monitoring programs are recommended.

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### Strengths and weaknesses of the use of functional traits in recent studies on macrophytes

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Key words: aquatic environment, functional traits, macroDIVERSITY, macrophytes

Macrophytes are getting increasing attention because of their multiple roles in aquatic ecosystems (Bakker et al. 2013). However, eutrophication and habitat degradation are threatening macrophytes globally (O'Hare et al. 2018). In this context, the use of functional traits (FTs), i.e. specific features measured at species/individual level, can give more quantitatively translatable information than the traditionally adopted taxonomic approach about their ecosystem functions (Phillips et al. 2016). This research aims to elucidate the use of FTs in recent macrophyte studies and is preparatory to the macroDIVERSITY project (PRIN 2017), which uses spectral and phylogenetic diversity to map functional diversity in macrophyte communities. A systematic literature review focused on aquatic plants and functional traits was carried out on SCOPUS database (www.scopus.com). The latest 53 papers, published in 2019, were examined. The main topics emerging from these works on macrophyte FTs relate to: 1) the role of FTs in shaping communities; 2) macrophyte responses to environmental gradients; 3) application in monitoring anthropic pressure; and 4) how FTs mediate biotic interactions. We recorded that 27 studies involve only single or few species and therefore results are hardly exportable at the ecosystem level. The most used FTs include leaf economic and morphology traits. We noticed a lack of attention on root traits and an extended use of physiological traits that are difficult to measure in the field and on larger scales. We therefore advise to promote collection and processing of root traits, and to push forward the integration of remote sensing and classical approaches to clarify the role of macrophytes at multiple scales.

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## Ecology and germination of orofite endemo-vicariants of Sicily

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Key words: environmental, geographical and taxonomic vicariates, germination, high-mountain, regeneration niche, seeds

Vegetal vicarious species, geographical or taxonomical, have a very similar ecology: the ones examined in this study are mountain species that grow on rocky soils at high altitude on Mount Etna and Madonie range, the highest mountains in Sicily. In this study, we examined their regeneration niche, focusing on their seed germination ecology. We verified if there are differences between our pairs of species and vicarious populations, in the response to different levels of pH, water availability and temperature, and if there is a correlation between our results and the environmental characteristics of the collection sites of seeds. The species examined are: *Anthemis aetnensis* Schouw ex Spreng. - *Anthemis cupaniana* Tod. ex Nyman. and *Erysimum etnense* Jord. - *Erysymum bonannianum* C. Presl pairs (different species within the same genus) and *Berberis aetnensis* C. Presl and *Cerastium tomentosum* L. (populations of the same species growing in two different sites). We used 1% Agar at 20 °C as control. The two taxonomic vicariates have the ability to adapt to a wide range of pH. The high pH negatively influenced the seed germination rate of some species. Regarding temperatures, all species have reflected the environmental conditions of the area where they live. The germination of the two taxonomic and geographical vicariates was inhibited by low osmotic potentials. The different seed germination responses of the studied species depend both on the species and on the ecological conditions of their growing sites.

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## Ethnobotanic Survey In Valmalenco (Sondrio). Research experience told through difficulties, human relationships and funny moments

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Key words: ethnobotany, Open Science, sustainable research, traditional uses

This ethnobotanic survey is underway in Valmalenco (SO) and is part of the Interreg V-A Italy-Switzerland project B-ICE (ID. 63143) (Bernina Terra Glacialis Project), which aims to create a new management model for the ongoing climate change and reach new sources for the local enhancement. In this context, a precious heritage is represented by the knowledge on the uses of spontaneous plant species. The survey is ongoing in the municipalities of Chiesa in Valmalenco, Caspoggio, Lanzada, Spriana and Torre di Santa Maria through the proposal of semi-structured interviews to the local communities. To date, 276 interviews were collected. These data highlighted that the most cited species were Achillea erba-rotta All. subsp. moschata (Wulfen) I. Richardson, Malva sylvestris L. and Vaccinium myrtyllus L., used in the therapeutic and food fields. The main forms of preparation were digestive infusions, liqueurs, smoothing baths and anti-inflammatory mouthwashes. The collected data were statistically analyzed using ethnobotanic indexes (ICF - Trotter, Logan 1986, FL, JI). Our investigation showed that traditional knowledge is still quite rich and alive in Valmalenco and that plants have an important role in the life of the local communities. Moreover, what is generally omitted are the difficulties encountered in human relationships: the wall of initial distrust that collapsed with a smile, the problems of communication, overcome with patience, the trouble of sharing spaces and managing everyday life among the members of a young research group. These aspects together account for an experience that combines traditional knowledge, science and humans, in a sustainable research perspective and in an Open Science context.

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## A multidisciplinary molecular approach for the conservation of the endangered *Orchis patens*

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Key words: Orchidaceae orchid conservation, orchid mycorrhiza, plastomes, population genetics, Rhizoctonia

Effective conservation science relies on species-relevant knowledge obtained from multidisciplinary research such as population genetics, mycorrhizal associations and pollinator interactions (Fay 2018). The Mediterranean *Orchis patens* Desf. (Orsenigo et al. 2016) is the flagship species of the European LIFE project LIFEorchids (LIFE17NAT/IT/000586, a project on the conservation of orchids and their habitats) because of an alarming decrease in the number of individuals in its natural populations. In this work, we used a molecular approach to study the causes of its rarity and its interaction with the other organisms of the ecosystem by means of the genetic diversity and structure with microsatellite markers and plastomes, and metabarcoding and isolation of mycorrhizal fungi. Our results are extremely important for the conservation of this species; the genetic data of *O. patens* suggest that subsp. *canariensis* should be considered a different species, therefore calling into question the global Red List Assessment. Furthermore, fungal metabarcoding results show the fungal specificity ("apparent generalism") that was already observed with closely related *Orchis* spp. (Jacquemyn et al. 2010), suggesting a high phylogenetic conservativism in the symbiotic interaction.

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## Orchids of Sardinia (Italy) as a model for biogeographic studies in insular conditions. A synopsis

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Key words: continental island, evolution, geographic isolation, Mediterranean basin, Orchidaceae, Sardinia

Biological and ecological investigations of islands are crucial to explain ecosystems functioning. Many studies on island biodiversity are carried out in oceanic islands. In contrast, information on continental islands such as those in the Mediterranean sea, are very often fragmented in space and time. Here, a synopsis of the Orchidaceae of Sardinia is presented, based on literature research and recent botanical explorations. Our final list comprises 62 species and 14 genera: thirteen species are recognized as endemic, three new species are recorded for the flora of the island and one was no longer found. This orchid richness reflects the geological history of the island that was linked to the mainland several times, facing long periods of isolation. We also discuss a critical point-of-view of the biodiversity shortfalls still problematic for orchids in island conditions. Indeed, within the Mediterranean basin, the greatest amount of endemism occurs mainly in large islands and, despite the long botanical tradition of European countries, many of them are scarcely investigated. This annotated synopsis shows the potential of continental islands to understand trends in ecology and evolution. Further studies are required to complete our knowledge of the orchid diversity on continental islands in order to propose scientific-based conservation programs to preserve these unique life-forms.

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## Betula aetnensis: thermal and water potential thresholds of regeneration by seed for a narrow endemic high mountain Mediterranean birch

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Key words: Betula aetnensis, climate change, endemic, germination, seedling development, thermal thresholds, water potential

Betula aetnensis Rafin. (Betulaceae) is an endemic tree of Mt. Etna (eastern Sicily), belonging to the Betula pendula Roth group. Nowadays, there are only two populations of B. aetnensis and their natural regeneration is severely declining (Strano 2010). Thus, it is important to understand its regeneration ecology in order to predict the plant response to changing climatic conditions. The influence of temperature and water potential on germination and seedling emergence has been widely demonstrated (Dürr et al. 2015). To verify if there is a difference in the temperature and water potential thresholds for germination among Mediterranean birches and the continental silver birch (Betula pendula Roth), we compared B. aetnensis with six populations of B. pendula sampled along a north-south gradient in Europe and a population of Betula fontqueri (Rothm.) G.Moreno & Peinado sampled in Spain. The thermal thresholds were calculated before and after 2 months of cold stratification treatment at 5 °C. Tests were conducted at six constant temperatures: 5, 10, 15, 20, 25 and 30 °C. The osmotic potential tested were -0.1, -0.2, -0.4, -0.6, -0.8, -1 and -1.5 MPa. It seems that both the base temperature and the base water potential for germination are more influenced by the local climatic variables then by the geographic distribution of the population sampled.

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## The role of protected areas and translocations in preserving Italian endemic species in the face of future climate change

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**Key words: c**onservation, endemic species, species distribution model

In the Mediterranean Basin, narrow ranges and specific ecological needs make most endemics prone to climate change. Consequently, translocations have become increasingly important in conservation worldwide for recovery of rare species and restoration purpose. Protected areas (PAs) are an essential tool for plant biodiversity conservation, preventing additional stress to plant populations and thus potentially buffering the effects of climate change, and may provide suitable sites for reintroductions. Nevertheless, their current boundaries will not necessarily be effective in the future. We performed Species Distribution Models (SDM) on 188 plants endemic to Italy and we calculated the future habitat suitability of their current populations under both an optimistic and a pessimistic scenario. Then we quantified the future suitable areas within PAs. SDMs forecasted that all the species will loss less than 30% of their current populations under the optimistic scenario. Conversely, under the pessimistic scenarios the majority of species will loss more than 50% of their populations; particularly 14 species will likely become extinct (i.e., populations loss higher than 80%). In around half of species, the majority of future populations will be outside PAs under both scenarios. Nevertheless, considering habitat suitability within PAs almost all species will potentially counterbalance the loss of populations through migrations. These results suggest that the current boundaries of PAs are poorly effective in mitigating the effects of climate change on Italian endemics. Nevertheless, they may be important sites for translocations to reduce the risk of extinction of endemic plants.

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## Management and conservation of Show caves: study of microbial biodiversity to evaluate the anthropic impact

F. Biagioli, C. Coleine, L. Selbmann

Key words: biodiversity conservation, fungi, management, show caves

The Show caves in Italy, in particular Karst-origin caves, represent an important naturalistic heritage. These hypogeum sites are peculiar and fragile ecosystems, due to their habitat conditions, spatial confinement, climatic stability and the conservation value of their cultural, geological and biological diversity. The impressive numbers of visitors (up to 500,000/year/cave) and the profits deriving from such activities have recently acquired importance at global scale, resulting in an increasing interest for the underground karst environments. Tourist flow and the associated transformations contribute significantly to alter the natural stability of the cave ecosystems. Despite the increasing interest, ecological consequences of the human-associated threats were mainly studied at local level, focusing on single biological components. In this frame, the PRIN "Showcave" project aims to assess the effects of anthropic impact, generated by tourists. The impact of cave tourism will be evaluated analyzing native and alien fungal biota in sediments and on speleothem surfaces, considering a gradient along the touristic paths. Fungal diversity will be assessed by both culture-dependent and -independent approaches, including cultivation, qPCR and metabarcoding sequencing. Expected results, such as identification of new taxa and indicator species of anthropogenic impact and pristine conditions, will expand our knowledge on mycobiota of karts caves and give us insights to preserve and manage integrity of these fragile ecosystems.

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## Conservation of endemic and rare vascular flora in the areas around Messina: current status and protection proposals

G. Tavilla, S. Cambria

Key words: biodiversity, conservation, Messina, Peloritani

The Peloritani floristic district, located in the North-Eastern part of Sicily, can be considered as one of the main biodiversity hot spots in the island, representing an important centre of speciation and plants refuge, due to its paleogeographic, geological, and bioclimatic features (Picone et al. 2003, Sciandrello et al. 2014). The municipal territory of Messina extends from the sea level (facing both the Tyrrhenian and Ionian seas) to Mount Dinnammare (1128 m a.s.l.), hosting a considerable environmental variety. The aim of this contribution is to provide an updated regional assessment on the conservation status of the rare and vulnerable flora of this area and at the same time to propose management measures for its preservation. The data concerning these species are presented: *Adenocarpus commutatus* Guss., *Anthemis messanensis* Brullo, *Anthemis peregrina* L. subsp. *peregrina*, *Artemisia campestris* L. subsp. *variabilis* (Ten.) Greuter, *Centaurea deusta* Ten. subsp. *divaricata* (Guss.) Matthäs, *Cistus crispus* L., *Fritillaria messanensis* Raf., *Hypecoum procumbens* L., *Linaria multicaulis* (L.) Mill. subsp. *multicaulis* var. *messanensis* Giardina & Zizza, *Osmunda regalis* L., *Tuberaria lignosa* (Sweet) Samp., *Viola messanensis* (W. Becker) Brullo, *Tricholaena teneriffae* (L. f.) Link. In particular, the areas located around Messina shows a particular floristic richness, including some very rare narrow endemic species. So, for example, *A. messanensis* was evaluated as critically endangered (CR). Instead, taxa that have here their only Sicilian or Italian population as *C. crispus* and *T. teneriffae* were evaluated endangered (EN).

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## Exploring the Diversity of *Psychotria* (Rubiaceae) in Lowland and Montane Rainforests in Papua New Guinea.

T. Jimbo, S. Sohmer, J. Wen

Key words: Diospyros, Momase, Papua New Guinea, Psychotria, Saurauia

The north eastern part of Papua New Guinea (especially Momase region) has a rich diverse flora that is still poorly known. We initiated a proposal to the National Geographic Society to explore forest of Papua New Guinea and establish the foundation for a molecular data in elucidating the evolutionary pattern and taxonomy of Papua New Guinea *Psychotria* L. (Rubiaceae). We conducted a short expedition into lowland forest of West Sepik (Kilifas, Utai, Finamui, Punda, Wamuru, Wutung) and montane forest of Morobe Province (Wagau) in 11th March – 6th April 2018, mainly focusing on the genus *Psychotria* and related taxa. A total of 218 collections (62 genera and 128 species) were made. Fifty-nine of that total collections are *Psychotria*, with eleven species identified to be endemic to Papua New Guinea. Some collections of *Psychotria* likely represents undescribed species. We found at least two possible new species, *Diospyros* sp. *nov*. (Ebenaceae) and *Saurauia* sp. *nov*. (Actinidiaceae). Our work suggests that many more new plant species and new records await discovery in Papua New Guinea. We submitted a report on this expedition to the National Geographic, and this paper is based on that Report.

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## Cryptoendolithic eukaryotic diversity of Helliwell Hills, Victoria Land (Continental Antarctica)

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Key words: Antarctica, cryptoendolithic communities, eukaryotic diversity, metabarcoding

In both cold and hot arid and hyper-arid environments such as the Antarctic ice-free areas, where epilithic colonization is not possible, microorganisms can find refuge inside rocks, forming the so-called cryptoendolithic communities, as last chance for survival. Previous studies revealed that these ecosystems, dominated by lichenforming fungi and black yeasts, are extremely adapted but particularly sensitive to any external perturbation (Selbmann et al. 2017, Coleine et al. 2018 a,b). Aim of this study was to analyze, for the first time, rock samples collected from three sites of Helliwell Hills (in the northernmost part of the Northern Victoria Land). Metabarcoding sequencing approach, targeting 18S region, was used to study eukaryotic diversity. 18S dataset was processed by AMPtk bioinformatic tool and global singletons and rare taxa were discarded; a 97% sequence similarity clustering threshold was utilized to identify the Operational Taxonomic Units (OTUs) using VSEARCH and SINTAX database. Preliminary results show a large predominance of Ascomycota in all samples, where Class Dothideomycetes prevailed, while Lecanoromycetes, Eurotiomycetes and Saccharomycetes were present in low abundance. Chlorophyta were mostly represented by algal species Trebouxia decolorans Ahmadjian. All the biodiversity indexes (Richness, Shannon and Simpson) confirmed that these ecosystems were composed of highly specialized microorganisms. This study revealed a significant difference between samples collected in Helliwell Hills and those from other localities previously analyzed in Victoria Land, suggesting that geographic distance and isolation influence diversity and community composition.

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# Molecular characterization and taxonomic revision of types of the genera *Xylaria* and *Rosellinia* belonging to the Pier Andrea Saccardo mycological collection

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Key words: DNA barcoding, Illumina sequencing, ITS, Rosellinia, type specimens, Xylaria

The fungaria represent a source of molecular information that can be exploited to enlarge the coverage of species-level DNA sequence information deposited in public databases (Osmundson et al. 2013). However, they are an underused resource for this purpose because obtaining DNA data from ancient biological material is extremely difficult (Pääbo et al. 2004). The Botanical Garden of Padova conserves the mycological collection of one of the most important mycologists lived in the 19th century: Pier Andrea Saccardo. This collection, dated 1874, is composed of 70,000 samples, but its scientific value is due to the presence of 4,000 types. These specimens have been borrowed by mycologists from all over the world for morphological revisions and consequent taxonomic reclassifications, but they have never been involved in sequencing projects. An Illumina sequencing was applied to obtain ITS (internal transcribed spacer) sequences, the barcode for the identification of fungal species (Schoch et al. 2012), from types of the genera Xylaria Hill ex Schrank and Rosellinia De Not. stored in the collection. This method was chosen to overcome the problems of the DNA fragmentation of the fungal samples and the presence of exogenous DNA contaminations (Forin et al. 2018). The molecular analyses not only have confirmed the current classification of many types, but they have also suggested that there is a need to reclassify others, demonstrating that in fungi the morphology does not always lead to a correct fungal systematic evaluation. These results show that, by applying modern molecular techniques, it is possible to obtain important information from ancient types useful to bring order in the fungal taxonomy.

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## Culture collection of Italian wood decay fungal strains for the development of mycelium-based materials

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Key words: culture collection, myco-materials, strains, wood decay Fungi

Myco-materials can be distinguished in two main big families: bio-composites (eg. building materials) and pure materials (eg. eco-leather). Mycelium in bio-composites is coupled almost exclusively with other (non-fossil) materials derived from biological processes, such as vegetable fibres, to exploit the natural growth of the fungal organism on these substrates. Fungi are able to give cohesion to incoherent materials due to the production of a mass of mycelium. The properties of myco-materials depend on substrate, type of fungus, its growth conditions and post-processing (Girometta et al. 2019). Mycelia of wood decay fungi (WDF) are now being explored for the production of this kind of materials. Up to now, only few species of WDF have been tested for bio-fabricated materials: Ganoderma lucidum (Curtis) P. Karst., Pleurotus ostreatus (Jacq.) P. Kumm., Schyzophillum commune Fr., Trametes versicolor (L.:Fr.)Pil. and Fomes fomentarius (L.) J.Kickx.f. A doctoral project at the University of Pavia is underway on this topic. The PhD project aims to select and characterize performing fungal strains with properties useful to one of the two families of myco-materials, so white mycelium (selected to obtain a dyeable final product) and rapid growth in relation to the cultural conditions will be preferred (Girometta et al. 2020a). During the first PhD year, about 130 WDF strains were obtained so the Fungal Research Culture Collection (MicUNIPV) of Pavia University (Italy) maintained up to now about 500 WDF strains. These strains were isolated from sporophores growing in different habitats and some of them are also useful for taxonomic and/or ecological studies (eg. Cellulariella warnieri (Durieu & Mont.) Zmitr. & V. Malysheva, Fomitiporia mediterranea M.Fisch., Fomitopsis iberica Melo & Ryvarden and F. officinalis (Vill.: Fr.) Bond. & Sing,, Perenniporia ochroleuca (Berk.) Ryvarden) (Girometta et al. 2020b).

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## Differences in taxonomic and ecological traits between Ehrharta erecta Lam. and Ehrharta delicatula Stapf (Poaceae)

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Key words: alien species, biodiversity, herbarium, invasiveness, vascular flora

Ehrharta Thunb. (Poaceae) is an African genus ranging from South Africa to Ethiopia and Yemen that includes approximately 35 species (Fish et al. 2015). Some species are alien in Australia, China, Indonesia, Mediterranean Basin, New Zealand, North and South America. Given their ecological and physiological characteristics, the species belonging to this genus are considered among the most invasive in the world. In the Euro-Mediterranean area four species are recorded (Valdés, Scholz 2018): Ehrharta calycina Sm. (Spain and Tunisia), Ehrharta delicatula Stapf (Italian peninsula), Ehrharta erecta Lam. (France, Israel, Italian Peninsula, Morocco, Netherlands, Portugal, Sardinia and Spain) and Ehrharta longiflora Sm. (Canary Islands and Spain). All these species show a high relative growth rate which has probably facilitated their naturalization outside their native ranges. Given that E. delicatula and E. erecta are reported for the Italian territory in the specialized literature (e.g., Pignatti 1982, Galasso et al. 2018), the aim of the work, based on bibliographic, herbarium and field research, is to highlight the differences between the two taxa in terms of taxonomic traits and ecological characteristics. On the basis of the herbarium samples analyzed, the morphometric measurements carried out, the analysis of the ecological characteristics and the analysis of the adaptive strategies adopted by the two species described in literature (Tilman 1988, Verboom 2000,, Grime 2001), we proceeded to provide new observations in order to correct the data present in the bibliography and thus allow the updating of the distribution map of the two species in Italy. The results of the study led to highlight some key features of the difference between the two species with consequent exclusion of *E. delicatula* from the Italian territory and therefore from the Euro-Mediterranean area.

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## Application of IUCN Red Listing Criteria at the Regional Levels: A Case Study with *Boletales* in Umbria (Central Italy)

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Key words: conservation, ITS1-ITS4, IUCN assessment, threats

The Boletales E.-J. Gilbert (Agaricomycetidae, Basidiomycota) is one of the major groups of mushroom-forming fungi that is represented in most forest ecosystems worldwide. Recently the intensive use of molecular tools applied to the study of the systematics of boletoid mushrooms and related groups has upset the traditional classifications based on morphological characters (Gelardi et al. 2019). At present, the Boletales has been divided into six suborders, about 19 families and 70 genera, grouping taxa with different morphology of fruiting bodies including poroid, gilled, resupinate, hypogeous and epigeous gasteroid forms (Gelardi et al. 2019). In this work, a Red List of all the *Boletales* recorded in Umbrian region (i.e., 79 species belonging to 36 genera and 11 families) is provided (Angelini et al. 2017). The conservation status was assessed according to the IUCN categories and criteria, adapted for sub-global level assessments (IUCN 2012a, b, c; IUCN 2017). The majority of species (72.12%) are classified as threatened, whereas 20.25% are Near Threatened (NT), 3.8% Least Concerned (LC) and 3.8% Data Deficient (DD). Inside the group of threatened species, no species are classified as Critically Endangered (CR), while 35.09% are Endangered (EN) and 64.91% are Vulnerable (VU). Conservation status varies among families and genera and some appear to be more vulnerable than others. For example, all the four species belonging to the Paxillaceae family are classed as threatened (100%); in the Suillaceae, six out of ten species are threatened (60%), a further two are non-threatened (20%) and two are DD (20%); in the Boletaceae, of the 51 species 38 are threatened (75.5%).

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## Critical issues in the compilation of a check-list of macrofungi

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Key words: check-list, fungal biodiversity, fungal taxonomy, Fungi

The study of macro-fungal diversity has undergone huge changes in the last decade, and the recent developments in molecular systematics have led to revolutions in taxonomy and to a proliferation of new taxa that are often not identifiable by morphology. Assembling accurate regional check-lists of macro-fungi is a task that can be crucial for red-lists and biogeographic studies; this requires to keep up with these fast taxonomic developments, applying new techniques to identify and separate the taxa occurring in the selected area, and it also requires to be able to re-evaluate the old data of occurrence under this new light. A new check-list of macro-fungi is currently being compiled for Liguria, a region of NW Italy notable for the richness of highly diverse habitats. Over 1000 new species are being recorded in Liguria, raising the total number of known occurring macro-fungal species to over 3000. This huge work gives us a chance to explore the critical issues and the new approaches to the compilation of check-lists of macro-fungi.

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## Influence of ants on lichens beta-diversity and functional traits

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The *Formica rufa* L. group comprises several ant species which are very important components of forest ecosystems. In fact, they are considered as natural engineers that may influence the structure and dynamics of the habitats they colonise. Due to their capacity to act as biological control agent, some *Formica paralugubris* Seifert nests, collected in the Alps, were transplanted to various sites along the Italian peninsula where they were formerly absent. It is well known how their capacity to control pests could influence other organisms like invertebrates (Frizzi et al. 2018) and cryptogams (Heinken et al. 2007). Nevertheless, information about the possible influence of *F. paralugubris* on lichens communities out of its native range, are not yet available. To fill this gap, we randomly selected six nests of *F. paralugubris* from the introduced populations as the center of six plot of 20 m of radius: three in the Campigna Biogenetic Nature Reserve and three in the locality of Abetone (Tuscany). In each plot six *Abies alba* Mill. located at increasing distance from each nest were chosen. On each tree trunk, 12 grids (one for cardinal point and at three different heights) of 10 x 50 cm were vertically attached and used to survey lichen. As control, we randomly sampled six plots of the same dimension (three in each location) in non-occupied areas. The presence of the ants seems not to affect lichens species richness but seems to influence some lichen functional traits, like the growth form. We also observed different pattern of beta-diversity among the colonized and not colonized sites.

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## Survey of culturable fungi in Antarctic cryptoendolithic communities in Victoria Land

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Key words: Antarctica, black fungi, extremophiles, Fungi, phylogeny

Endolithism represents a borderline adaptation for microorganisms under the most extreme conditions of both cold and hot deserts (Selbmann et al. 2017). In the ice-free areas of continental Antarctica, where the conditions approach the limits to support life, self-supporting microbial endolithic ecosystems represent the main life-form living in a weak balance between life and extinction (Frieddmann 1982, Zucconi et al. 2016, Selbmann et al. 2017). Fungi are invariably present in these ecosystems that represent a reservoir of new and extremely adapted taxa. This work is based on a comprehensive sampling of sandstones collected in 26 localities in the Victoria Land (Continental Antarctica), ranging from 834 to 3100 m a.s.l., in order to investigate variation in biodiversity and composition of cryptoendolithic fungi. Culture-dependent analysis revealed *Friedmanniomyces endolithicus* Onofri and *Extremus antarcticus* (Selbmann & de Hoog) as the most abundant species among black fungi, known as an ecological group, specialized in the extremes; in particular, the Antarctic endemic genus *Friedmanniomyces* spp. was found in almost all rock samples analyzed. Lichenized fungi were mostly represented by species belonging to the genera *Acarospora*, *Lecanora* and *Lecidea* (*Lecanoromycetes*). Molecular phylogeny based on ITS region revealed the presence of three new species in the genus *Friedmanniomyces*, one new *Rachicladosporium* species, one new *Cryomyces* species; two new genera and four new species were found in in the Teratosphaeriaceae.

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## Coexistence of intensive rice production and *Marsilea quadrifolia* in the organic rice farms of the Po Valley

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Key words: Marsilea quadrifolia, rice production, translocation

Agricultural activities are one of the main factors driving biodiversity loss (Thorn 2016). Among these, rice cultivation caused considerable environmental impacts (Fusi et al. 2014), mainly because of water eutrophication and massive herbicides use (Capri, Karpouzas 2007). However, the recent introduction of sustainable cultivation practices has improved the environmental conditions of paddy fields, suggesting the reintroduction and survival of threatened species in agricultural areas. We tested whether rice production and plant conservation can coexist in the Province of Pavia, one of the most important areas for rice production in Italy. We focused on Marsilea quadrifolia L., an aquatic pteridophyte previously considered a weed in paddy fields and currently listed as "Endangered" in the Italian Red Lists (Rossi et al. 2016) and protected at EU level (Bruni et al. 2013) because of intensive agronomic techniques (e.g., use of herbicides, simplified rotation). Interestingly, recent occurrences were found in organic paddy fields, likely as a consequence of improved environmental conditions. Aiming to understand what environmental conditions may affect the species performance, an experimental cultivation was conducted in 2017 and in 2018 in eight rice paddies with different farming systems (organic, in transition and conventional farms). Six 20 × 20 cm potted swards of *M. quadrifolia* were placed along a transect in each field. The species best performed in organic farms, reporting the highest biomass in both years, whereas in conventional farms plants did not survived. These results will help us to identify the most suitable conditions favoring survival and spread of the species in rice fields, suggesting its future recover in agricultural areas.

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## Proposal of Pest Risk Analysis (PRA) application within limited territories

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Key words: conservation of Natura 2000 sites, invasive alien species, PRA

Invasive alien plants (IAS) are non-native species, generally introduced by man, able to spread quickly over extended areas by vegetative propagation and by seed, threating biodiversity, landscape structure, ecosystem functions, economy and human health (Szymura et al. 2016). The European Union adopted regulations aimed at listing, preventing and managing IAS introduction and spread, while the EPPO developed the Pest Risk Analysis (PRA), a protocol addressed to assess IAS potential risk and phytosanitary measures to mitigate them, commonly applied in EU member states or in the EPPO region (FAO 2007, EPPO 2012, EU 2014, 2016, 2017, 2019). However, this approach may underestimate the potential risk at local level. Therefore, we tested the application of PRA in Riserva San Massimo (462 ha~), SCI IT2080015 (Gropello Cairoli, PV - Italy), that hosts a black alder wood (habitat 91E0\*) in which populations of Solidago gigantea Aiton and Amorpha fruticosa L. have been surveyed. IAS population density and coverage were detected according to a stratified-random method (Huebner 2007). Results confirm that *S.gigantea* and *A.fruticosa* mostly invade the habitats where native species competition is lower and anthropic disturbance intense. The high naturalness and biodiversity of the habitat 91E0\* effectively counteracts IAS spread within itself, where vegetation is thicker and anthropic disturbance low. IAS population density is medium limited in wood clearings and at its edges. However, Riserva San Massimo plant biodiversity is seriously threaten by *S.gigantea*. PRA application in a limited area represents a valid tool to determine the level of risk of invasion associated with alien species and to update the Reserve Management Plan actions against them.

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- UE, Regolamento di esecuzione 2019/1262 della Commissione del 25 luglio 2019 che modifica il regolamento di esecuzione (UE) 2016/1141 per aggiornare l'elenco delle specie esotiche invasive di rilevanza unionale.

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# Discovery and Characterization of *Pycnoporellus fulgens* from an unmanaged forest in the Biogenetic Nature Reserve of Vallombrosa within the LIFE MycoRestore Project

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Key words: biocontrol, environmental mycology, LIFE Mycorestore, Pycnoporellus fulgens

Unfavorable growth environments generated by climate change can alter the equilibrium between a pathogen's virulence and the number of trees which are able to complete their life cycle without any serious interference from the pathogen. In this context, the LIFE MycoRestore (LIFE18/CCA/ES/001110) project combines forestry practices and mycological resources, aimed at increasing the resilience of Mediterranean forests in selected areas of Italy, Spain and Portugal, by a) identifying mycorrhizal/non-mycorrhizal fungi that characterize healthier environments and which are known biocontrol agents and b) introducing or boosting up them in the environments which are mostly affected by biotic and abiotic stress. In Italy, the forest of the Biogenetic Nature Reserve of Vallombrosa (Tuscan Apennines, Florence province) has been chosen as a demonstrative site for this project. Here, wilting and death of whole chestnut (Castanea sativa Mill.) and silver fir (Abies alba Mill.) trees is often observed as a consequence of ink disease (Phytophthora cambivora (Petri) Buisman and P. cinnamomic Rands), and root rot (Heterobasidion abietinum Niemelä & Korhonen and Armillaria ostoyae (Romagn.) Herink), respectively. A preliminary, morphology-based screening of sporocarp-producing fungi in soil around healthy and symptomatic trees suggested a reduction in species richness in the latter condition. Further assessments in this direction will be made through metagenomics in soil, wood and leaf samples coming from healthy and diseased plants. A first indication of this hidden fungal diversity comes from the sampling, from an unmanaged sub-area characterized by tree falls, of *Pycnoporellus fulgens* (Fr.) Donk, a rare polypore. A phylogenetic analysis will clarify whether this isolate relates with samples from other environments.

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## The biodiversity of soil microfungi in Colombia

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Key words: biological diversity, Colombia, Fungi, soil, South America

Although Colombia is one of the first tropical countries where an effort was made to gather a mycological flora, contributions to the taxonomy, diversity and ecology of soil microfungi are still scarce. In this study, the diversity of soil microfungi was analysed collecting biodiversity data from literature and dividing them into the five natural regions of the country: Andean, Amazonian, Caribbean, Orinoquía, Pacific and Insular. The analysis showed that most data come from the Andean region, while the other regions were much less studied, with the Insular one that was not studied at all. This may be because the Andean region is the most populated one, and thus the most accessible to research activity. No more than 170 species of soil microfungi were recorded for Colombia, belonging to 58 different *genera* and 3 *phyla* (*Ascomycota*, *Zygomycota* and *Glomeromycota*). Referring to the soil fungi of the country, arbuscular mycorrhizae represent the most widely studied group. The data from this review allow to affirm that the most recorded *genus* in Colombian soils was *Glomus* with 38 species, followed by *Acaulospora* with 13 species, *Penicillium* with 12 species, *Aspergillus* and *Fusarium* each with 10 species in the soil. Therefore, it is evident the need to continue and complement the existing research on microfungi in Colombia to have a better ecological understanding of soil and its properties.

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## Ptilostemon casabonae (L.) Greuter: phytochemical and biomolecular intra- specific variability of a little known endemic Mediterranean plant

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Key words: biomolecular analysis, endemism, HPLC-PDA-MS/MS, intra-species variability, P. casabonae (L.) Greuter

Ptilostemon casabonae (L.) Greuter is a Mediterranean endemism localized in Sardinia, Corse and Hyères islands (France), where it is traditionally used for its healthy properties. The species is widespread in its natural habitats therefore it is not currently considered an endangered species (Atzei 2003, Marengo et al. 2015). This work aims to add information on P. casabonae, through a combined fingerprint based on phytochemical and biomolecular patterns. Several individuals were collected from three different sites, two from Sardinia (Italy) and one from Corse islands. The hydroalcoholic extracts of *P. casabonae* aerial parts were investigated here for the first time through HPLC-PDA-MS/MS analysis, resulting in flavonoids and phenolic acids as main components. Samples from the three sites showed similar phenolic profiles, although statistical analyses highlighted some quantitative differences for some compounds. The amplification and sequencing of ITS, 5S-rRNA-NTS, and psbA regions did not reveal nucleotide differences among *P. casabonae* samples from different geographical origins. A comparison with other Ptilostemon species sequences, from Genbank, revealed an inter-species variability of ITS and psbA regions (Marengo et al. 2019). The stability of both the phenolic and the biomolecular profiles within *P. casabonae* allowed us to identify a set of specialized metabolites that can be adopted as biomarkers and useful specific DNA regions to distinguish it unequivocally. The combination of the phytochemical and biomolecular data provides a useful fingerprint on *P. casabonae*, able to depict this little-known plant for future investigations.

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## LIFEorchids (LIFE17NAT/IT/000596): propagation and dissemination

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Key words: conservation, germination, Orchidaceae, orchids, seeds, social networks

Despite being among the most species-rich plant communities, semi-natural grasslands (such as habitat 6210\*) are among the most threatened in Europe due to their sensibility to land use change. LIFEorchids (LIFE17NAT/IT/000586) is a project on conservation of orchids and on safeguarding of their habitats in Northwestern Italy (www.lifeorchids.eu). Indeed, some orchid species are extremely rare and/or endangered (Dostalova et al. 2011, Rankou 2011, Calevo et al. 2018). The project, involving three research institutes, two managing bodies, and two non-governmental associations committed to environmental protection, aims at conserving orchids and their habitats, i.e. by developing protocols of propagation and reintroduction for the targeted orchid species, but also by implementing public awareness through dissemination and the use of social networks. In this work, we present preliminary results concerning germination trials of selected orchid species targeted by the project as well as isolation of orchid mycorrhizal fungi (OMF); i.e. we isolated the OMF Tulasnella helicospora Raunk. from the roots of Orchis patens Desf., flagship species of the project, confirming its compatibility for seed germination. Furthermore, we analysed the public interest on social networks to understand the topic effectiveness and to estimate the suitability of the tools used (i.e. the videos length). Short videos (max. 1.30 mins), as well as photos of plants and people, were the most viewed especially during the morning and late evening. This analysis represents an important starting point for conservationists allowing them to understand how to adequately disseminate conservation outputs in the view of improving public awareness.

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## Drupe development in different plum species

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Key words: cell expansion, cell division, drupe, ovary

The mechanisms driving drupe differentiation have been investigated in several plant species. We aimed to investigate whether, in three Prunus species (P. salicina Lindl., P. domestica L., P. cerasifera Ehrh.), fruit size is related to ovary size or whether it depends on post-bloom developmental processes. We used microscope image analyses to evaluate ovary wall thickness at anthesis and fruit mesocarp thickness at ripening. In the same tissues, we also measured cell area and number of cellular layers. Ovary wall thickness did not differ among the species tested, except for *P. cerasifera*, the smallest-fruited genotype, having bigger ovaries. Fruit mesocarp thickness was strongly positively correlated to the number of cell layers, weakly negatively correlated to cell size. No correlation existed between fruit weight and the respective ovary characteristic: bigger fruits did not derive from bigger ovaries. Fruit weight was instead related to the fruit-to-ovary weight and cell layer number ratios, indicating that fruit size in these species is related to processes that occur to a different extent after bloom, particularly to a greater cell division. In Rosaceae, sorbitol and sucrose are major sink-translocated forms and their metabolism can be responsible of these differences. Thus, we looked for the annotated sequences of seven genes related to their metabolism. They are only available for P. persica (L.) Batsch., which has been used to design primers; we sequenced the PCR products and designed new specific primers for our plum species. Aiming to highlight differences in expression extent and transcript localization, real time RT-PCR and in situ hybridization are in progress.

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## New molecular insights on the *Tuber aestivum-mesentericum* speciescomplex

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Key words: aroma, ascocarp, phylogenesis, truffle, Tuber mesentericum

Tuber aestivum Vittad. is one of the most marketed truffles in the world. It is morphologically similar to Tuber mesentericum Vittad., a truffle only locally appreciated (Italy, France). Because T. aestivum and T. mesentericum have very similar ascocarp features and *T. aestivum* var. *uncinatum* Chatin is collected in similar environments and periods of T. mesentericum, they are frequently mistaken with one another. Forty-three T. aestivum and T. mesentericum ascocarps were collected all over Italy for a morphological and molecular characterization. According to their morphological features, three samples were classified as T. mesentericum, although the aroma of the fresh specimens showed complete absence of the phenolic note. DNA was extracted from 27 samples and the elongation factor  $1-\alpha$  (EF1 $\alpha$ ) locus sequenced. Obtained sequences confirmed the taxonomic association to T. aestivum or T. mesentericum and were used for building a Maximum Likelihood phylogenetic tree. The tree showed that T. aestivum and T. mesentericum sequences cluster into different clades, with T. mesentericum sequences divided into three different sub-clades. In particular, sub-clade III showed the lowest genetic distance from T. aestivum clade. This study reveals a high genetic variability of T. mesentericum in the EF1 $\alpha$  locus. We identified a well-defined T. mesentericum sub-clade for the specimens characterized by a pleasant aroma similar to that of *T. aestivum*. Moreover, we confirm that discriminating *T. aestivum* from *T. mesentericum* ascocarps can be difficult, pointing out that the existence of ecomorphs with pleasant aroma can represent an opportunity to improve the market of this truffle.

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## Mapping coastal dune natural vegetation types using a measurement of phenological features: the potential of Sentinel-2

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**Key words:** coastal monitoring, dune vegetation classification, multi-temporal classification, remote sensed phenology, Sentinel-2

Coastal dune landscapes are heterogeneous mosaics hosting highly specialized biodiversity which mapping and monitoring represent a challenge. The increasing availability of free remotely sensed data offers good bases for developing effective monitoring tools. We explored the potential of the multitemporal Sentinel 2 imagery for mapping coastal landscapes at two levels of detail: i) main land cover types and ii) vegetation mosaics (sensu 92/43/EEC). We also investigated its potential for mapping an invasive alien species (Acacia saligna (Labill.) H. Wendl.). The test area includes representative tracts of the Adriatic (Molise) and Tyrrhenian (Lazio) coasts in central Italy. Using Sentinel 2 imagery (years 2016-19) we analyzed the monthly behavior throughout the year of: Normalized Difference Vegetation Index (NDVI), Modified Soil-Adjusted Vegetation Index 2 (MSAVI2) and Normalized Difference Water Index (NDWI), as proxies of vegetation phenological proprieties and water seasonality. Images were analyzed using free software made available by the European Spatial Agency (ESA). We preprocessed images using sen2cor processor and implemented a hierarchical classification using random forests algorithm (Sentinel Application Platform-SNAP). We performed accuracy analysis using aerial photos and field surveys. The obtained classification adequately describes coastal dune mosaic, with high accuracy for the higher level of detail (land cover) and intermediate for fine scale vegetation mosaics. The attempt of mapping the A. saligna invaded patches also presented good levels of accuracy. Multitemporal Sentinel classification seems an effective approach for mapping coastal dune mosaics. Our results suggest the possibility of extending the proposed procedures for mapping wider coastal areas and for mapping other heterogeneous landscapes.

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## An updated checklist of the vascular flora of Montagna di Torricchio State Nature Reserve (Marche, Italy)

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Key words: alien plants, Central Apennines, endemisms, floristic diversity, Herbarium CAME, nature conservation

This study aims to increase the floristic knowledge of the Marche Region by means of a floristic investigation conducted in the State Nature Reserve "Montagna di Torricchio" (Marche, Italy). The Reserve is located in the Central Apennines, it is extended for about 320 hectares and ranges from 820 to 1,491 m a.s.l. It is owned and managed as a strict reserve by the University of Camerino since 1970: all the anthropic activities (agriculture, semi-extensive breeding and forest cutting) ceased about 50 years ago, except for a very small area, where mowing and cow grazing are still allowed (Ballelli, Francalancia 1982, 1987). The present floristic checklist is composed of 789 taxa at the species and subspecies level (352 Genera, 81 Families), 127 taxa more than the previous floristic surveys (1987). Two taxa were found to be new for Italy (*Taraxacum calocarpum* Sonck. and *Taraxacum pulchrifolium* Markl.) and 14 for the Marche Region, 46 were endemic to Italy while only 11 species were alien. With respect to the previous survey allowed (Ballelli, Francalancia 1982, 1987), we showed a stability in the life-form spectrum, suggesting limited effects of dynamic processes related to climate and land use changes. In particular, the negligible number of alien species is probably related to the weak impact of the anthropic influence, mainly ceased 50 years ago. Additionally, the new species reported for Italy and for the Marche Region highlight the importance of the Reserve for species conservation in the context of Central Apennines.

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## How lichen functional traits vary along a latitudinal gradient in Chile?

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Key words: Chile, community ecology, functional traits, latitudinal gradient, lichen

The study of inter- and intraspecific functional trait variability provides us an interesting approach to predict the capacity of communities to cope with climate change and acclimate to future environmental conditions. Even though there are detailed protocols for measuring functional traits in other groups of organisms, mainly vascular plants, little is known in lichens related to their functional traits. For this reason, we have evaluated the interand intraspecific variability of different functional traits in more than 100 macrolichen species growing on *Nothofagus pumilio* (Poepp. & Endl.) Krasser forests along its distribution range in the Southern Hemisphere. To find the drivers that are behind this functional variation, we sampled 24 forests across a latitudinal gradient in Chile with a broad variety of climatic conditions. We collected five thalli of each macrolichen species found within each forest and we measured different functional traits, such as the specific thallus mass (STM) and the water holding capacity (WHC). Preliminary results indicate that these 'hard' functional traits related to the water use strategy were influenced by easily measurable 'soft' functional traits (e.g. growth form and photobiont type). Besides, we found significant effects of climatic drivers shaping the response of these functional traits. Temperature related variables (temperature seasonality and minimun temperature of the coldest month) are positively related with the STM, while precipitation related variables (precipitation of the warmest quarter) negatively affected the WHC.

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