PROCEEDINGS OF THE 50TH ANNIVERSARY CONFERENCE OF THE MASSANE FOREST NATIONAL NATURE RESERVE

Forest dynamics, yesterday, today, tomorrow

SELECTION PROCESS FOR WORLD HERITAGE SITES OF "ANCIENT AND PRIMEVAL BEECH FORESTS OF THE CARPATHIANS AND OTHER REGIONS OF EUROPE".

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The current extent of the serial transnational World Heritage Property of the Ancient and Primeval Beech Forests of the Carpathians and other regions of Europe consists of 93 component parts in 51 management units (protected areas). The designation process was done in 4 steps, starting with the first inscription of components in Ukraine and Slovakia in the year 2007 and the first extension by 5 German sites in 2011.After this first phase, a screening process was started 2012-2014 to elaborate a scientifically based overview on the best examples of postglacial beech forest expansion across Europe. This led to two further extension processes 2016-2017 and 2019-2020. The strictly protected beech forest of Massane had been included in the serial property in this last extension.Each possible candidate for an extension of the existing World Heritage Property needs to contribute a significantly additional value to the Outstanding Universal Value, that builds the main criterion for the inscription into the World Heritage List.The selection process was based on the existing knowledge on protected primeval and old growth beech forests in Europe and took into consideration the size of the potential sites, the protection status, the time since last management impacts, the climatic Beech Forest Region and local site factors like geology or elevation.The screening of literature and several expert meetings resulted in a long list with about 120 protected Beech Forests in Europe out of 23 countries. Based on the long list, an expert selection based on the criteria mentioned above led to a subsample, the "Vienna Short List" with large, strictly protected beech forests in about 50 protected areas. This list gave the framework for the selection of possible sites for the extension process of the World Heritage Property.Not all possible sites had been selected for a nomination because of individual decision of States Parties regarding their participation.As the knowledge on distribution of primeval and old growth beech forests increased in the last decade, new sites have been added to the long list and some of them had been nominated and inscribed in the extension in 2016 and 2019.The understanding of the screening and selection process is important to understand the existing network of this complex European World Heritage Property and the options and limitations to future extensions.

HOW TO ASSESS THE LEVEL OF SENESCENCE IN BEECH FORESTS? THE CASE STUDY OF THE UNESCO WORLD HERITAGE BEECH FOREST NETWORK.

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The European Union biodiversity strategy for 2030 aims to identify, map, monitor, and strictly protect all primary and old-growth forests still present in the EU. The Commission recently published a guidance note containing definitions, but there is still some confusion and debate about how to delimit these forests.In this presentation, we explain the difference between so-called ‘primary’, ‘old-growth’ and ‘senescent’ (over-aged, over-mature) forests.We also clarify that the concept of senescent forest is not a binary status (yes/no), but rather a gradient of maturity levels for a wide range of possible indicators.In order to delimit and protect senescent forests, it is necessary to set thoughtful threshold values and combine them in this set of gradients. These threshold values must be based on reference values and ranges.The UNESCO World Heritage Site “Ancient and Primeval Beech Forests of the Carpathians and Other Regions of Europe” is a transnational site, composed of 93 constituent elements, spanning 18 countries and including some of the best examples of primeval and senescent beech forests in Europe.As part of the LIFE-PROGNOSES project, we sampled a wide range of stand parameters related to senescence characteristics in a subset of the World Heritage Site and its buffer zone, in order to cover both the most “natural” beech forests and a wide range of maturity levels.Here we present the first preliminary results of our analysis, determining a wide range of indicators and their reference values, as well as how to combine, synthesize, and use them.

REGENERATION OF THE MASSANE BEECH FOREST, FROM FLOWER TO SEEDLING.

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The Massane National Nature Reserve possesses a wide variety of forest cover in terms of composition and morphology, which is the result of past practices and forest management. Although it is currently dominated by beech trees, its structure and composition are likely to change in response to climate change. Located at the southern limit of the beech tree's range, the Massane forest plays a key role in studying the response of temperate forest ecosystems to global warming.In order to study the different stages of forest regeneration in the Massane reserve, comprehensive long-term monitoring has been carried out since 2016 as part of the OSU-OREME's (Observatoire de Recherche Montpelliérain de l'Environnement) observation tasks for the main woody species in the reserve. This allows the different phases of production (leaves, flowers, seeds) to be monitored, as well as seedling germination and survival. To this end, 35 litter collectors have been installed within the integral reserve (IR), as well as 30

permanent plots distributed within the IR(18) and outside the IR (12).Since 2016, three years of massive production of beechnuts have been observed. We present the initial findings that provide a better understanding of the factors triggering fruit production years, their intensity, and ultimately their effectiveness for regeneration within different stands.

WHAT FUTURE FOR BEECH TREES AT LA MASSANE? A MODELING APPROACH.

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The European beech *Fagus sylvatica* is a species that bears witness to climate change and its consequences on French forests. It is indeed the second most sensitive forest species to rising temperatures and the increased intensity and frequency of droughts in France. Models predict a sharp decline of this species at the southern limit of its range by the end of the century, particularly in Occitanie. In order to better predict the speed at which these changes will occur and to study the relevance of forest management practices involving assisted migration, it is necessary to disentangle the relative weight of the various causes involved in tree mortality and to identify the factors of resilience. While the role of phenology in adaptation to climate change has been addressed many times from the perspective of phenotypic plasticity, the role of its intra-population variability on the resilience capacities of populations remains poorly studied. The phenology of beech is unique because of its sensitivity to day length, in addition to its sensitivity to temperature like all other species. How photoperiod interacts with temperature to regulate beech bud break is still poorly understood, and the consequences of this specificity on the resilience of beech to climate change have not been studied. In this study, conducted as part of the FAGADAPT project of the BiodivOc program, we investigated the impact of bud break date on beech growth and fitness under historical and future climate conditions in order to identify whether a particular phenotype would be better suited to future conditions. Indeed, beech trees in the Massane nature reserve show significant variability in bud break date. We used the PHENOFIT model to simulate the performance of beech trees from 1950 to 2100 across the Massane nature reserve. We first developed a bud break date model that takes into account the effect of day length. We calibrated this model and other existing models using observations made at La Massane. By reproducing the phenological variability observed at La Massane between early-budding and late-budding individuals, we show that this trait is responsible for significant differences in fitness. Regardless of the phenological model used, early individuals perform better than late individuals on average, and the gap is expected to widen in the future according to projections. The fitness landscapes calculated with PHENOFIT reveal a selection for early bud break under historical and future conditions if global warming does not exceed 2.5°C, but with average fitness decreasing sharply at the bottom of the altitude gradient. Above 2.5°C, selection no longer appears to be unidirectional, weakens, and fitness continues to decline across the gradient. The future of beech trees at La Massane therefore seems to be highly dependent on the success of government policies to limit global warming to 2°C.

EXPLORING LIFE

TREE-RELATED MICROHABITATS AS KEY STRUCTURES FOR BIODIVERSITY

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Tree-related microhabitats (TreMs) are morphological features found in certain trees, both living and dead, which constitute discrete and temporary pockets of habitat resources. They contribute to the high structural heterogeneity of forest ecosystems. TreMs are particularly numerous and diverse in forests that contain large, old living trees as well as dead trees. Several thousand species benefit from the presence of TreMs, covering a very wide range of taxonomic groups, from arthropods to mammals. The study of the relationships between TreMs and associated species has so far focused mainly on insects, birds, bats and amphibians. Very few studies have taken into account meiofauna (nematodes, rotifers, platyhelminths, tardigrades, and gastrotrichs), which nevertheless play a crucial role in the functioning of forest ecosystems. To begin filling this gap, we conducted an exploratory study on a wide range of TreMs borne by beech in the long-term unharvested Massane National Nature Reserve.

DISTRIBUTION, DIVERSITY, AND ECOLOGICAL ROLE OF MICROINVERTEBRATE COMMUNITIES INHABITING THE BEECH TREES OF LA MASSANE.

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The old beech trees in the Massane National Nature Reserve are home to a remarkable diversity of Tree-related microhabitats (TreMs), morphological features that serve as places of nutrition, reproduction, and hibernation for many organisms. These TreMs can either be carried by the tree (epiphytic TreMs, e.g., mosses, lichens), or include decaying wood (“saproxylic” TreMs, e.g., soil cavities, woodpecker lodges), or take the form of perennial or ephemeral fungal structures (e.g., sporophores of tinder fungus). TreMs have been classified based on their morphological characteristics and the presence of characteristic species assemblages at the macroscopic scale. Here, we focused on the microscopic fauna (nematodes, rotifers, tardigrades, insect larvae) inhabiting these TreMs, hypothesizing that the assemblages of species of this “microfauna” should correspond to the current typology of TreMs. This would enable us to identify indicator assemblages on a finer scale, but also, given the varied ecological traits of the species in place, we should be able to better understand the role of TreMs in the functioning of the forest ecosystem.The results show that TreMs are a real hotspot in terms of abundance, with, for example, abundances ranging from 100 to over 1,000 nematodes per gram of dry substrate, which is much higher than the abundances generally measured in all types of soil. The diversity of nematode fauna is considerable (99 morphological species, more than 1,000 when sequences based on DNA fragments 185 and 285 are added). Epixylic TreMs are

particularly rich in species and functional groups. The carbon and nitrogen isotopic signatures of the invertebrates that live there highlight the existence of complex food webs dominated by tardigrades, mites, and omnivorous and predatory nematodes of the Dorylaimidae and Mononchidae families. Saproxylic TreMs are dominated by a detrital loop and bacterivorous nematodes of the Rhabditidae family, which can reach extreme abundances there. The case of woodpecker lodges is quite unique, as the isotopic signatures reveal a trophic pathway that relies mainly on nitrogenous guano inputs from woodpeckers. The sporophores of perennial or ephemeral lignicolous fungi harbor a mainly fungivorous fauna, which is therefore likely to have a direct effect on the distribution and development of these saproxylic fungi in the beech forest.The overall results of this exploratory study provide arguments for the management and conservation of biodiversity in forest ecosystems, in that it appears that trees bearing TreMs are refuges for abundant and diverse microfauna and support complex food webs that are involved in important ecosystem functions at the forest scale.

THE ASILIDAE OF LA MASSANE: REVISION OF THE SPECIES LIST AND TAXONOMIC CLARIFICATION.

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The Asilidae of La Massane have been the subject of two previous studies. The current revision of the list of Asilidae in France will profoundly change our knowledge of this family of Diptera. The collections containing Asilidae from La Massane have been revised, invalidating 18 taxa from the previous list. Three species new to science were found in the Massane boxes, including two that come from the Massane Reserve. Holopogon garriguei sp. nov is named in homor to Joseph Garrigue in the revision of France.

THE HERITAGE LICHENS OF THE MASSANE FOREST RESERVE.

A UNIQUE TESTING GROUND FOR UNDERSTANDING THE BACTERIAL DIVERSITY ASSOCIATED WITH LICHENS.

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In recent years, while the presence and role of non-photosynthetic bacteria in the lichen symbiosis has increasingly been recognized, many questions remain regarding the identity and breadth of a core microbiome and the influence of different environmental factors, and of lichenizing fungal phylogeny, on the lichen microbiome. The Reserve de la forêt de la Massane represents a unusual old growth forest habitat that has been in free evolution for 150 years, and that due to its protection status and long term represents an rare opportunity to study lichen-microbiome interactions. In four dates roughly covering four seasons from 2022 to

2023 we sampled triplicate lichens from 5 different genera (Ramalina, Parmotrema, Lobaria, Anaptychia, Flavoparmelia) and 7 different species, inhabiting different substrates (Beech, Oak, Rocks) and at different heights. Whenever possible, samples from the same lichen individual were obtained in the four samplings. Bacterial community diversity was studied by metabarcoding of 16SrRNA genes in parallel with identification of the fungal symbiont by ITS-5.85 RNA sequencing. Samples were also use to selectively isolate organisms of interest. These data were used to test the following hypothesis 1) A core microbiome common to all lichens in all samples exists 2) A specific core microbiome specific for each of the species in all samples exists 3) Phylogenetic relationships of the fungal symbiont are more important than substrate and season on the bacterial community composition. We will present and discuss results of these analyses.

AN OPEN-AIR LABORATORY

POCKET-SIZED MINERAL DIVERSITY IN THE MINERALIZED PEGMATITES OF LA MASSANE

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The Massane Forest National Nature Reserve and its surroundings rest on a subsoil consisting of schists intersected by veins of granitic rocks (leucogranite, pegmatite, etc.) dating back to the ancient Variscan mountain range more than 300 million years ago. The regional geological structure is characterized by the metamorphic dome of the Albera Massif, whose core exposes migmatites from the ancient, partially melted Variscan crust, while the eastern edge exposes the high-temperature, low-pressure metamorphism gradient recorded in the schists from the Vall gorges to the Mediterranean coast. The schists at the edge of the dome are cut by multiple pegmatite veins, some of which are notable for the presence of numerous phosphate mineralizations. These veins form the mineralized pegmatite field of the eastern Albera. The mineralization in these veins consists of multiple species of micro-minerals, crystallized in small geodes generally concentrated at the edge of a vein. The total mineral diversity recorded in these veins is 90 species, including 57 species of phosphates, and features numerous microcrystals of very aesthetic appearance with few known equivalents on a national scale. Two areas of mineralized pegmatite fields are located in the immediate vicinity and within the boundaries of the Mas-sane Forest Regional Nature Reserve. The Font Andreu vein, located north of the reserve, is characterized by a unique mineral diversity, consisting of numerous species of zinc- and iron-rich phosphates that are unique within the pegmatite field. The second group, partially located within the reserve and recently discovered, has not yet been analyzed in detail, but initial results show a mineral diversity probably comparable to that found in veins in the Collioure area, further to the northeast. The interest of this geosite and the richness of its mineralogical material have led to it being submitted to the Regional Geological Heritage Commission for future inclusion in the National Geological Heritage Inventory.

ENVIRONMENTAL DNA METABARCODING: A COMPLEMENTARY TOOL FOR CHARACTERIZING FUNGAL DIVERSITY IN THE SOILS OF THE MASSANE RESERVE?

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Environmental DNA metabarcoding has become a tool for inventorying multiple substrates, including water, air, dust, and sediments, but it has been particularly revolutionary for the study of fungal biodiversity hidden in soils. These new tools make it possible to address their taxonomic and functional diversity, and question their ecology in sites where soils are particularly well preserved, such as old-growth forests and ancient forests. In the context of the UNESCO nomination of the Massane reserve, we characterized the fungal biodiversity using environmental DNA metabarcoding on soils, using Miseq technology and targeting ITS1 barcode and compared it with previous inventories. A total of 12 one-hectare plots were selected, representing a gradient of maturity, and 192 soil samples were collected and sequenced. Up to 3,642,843 sequences were produced and 11,932 operational taxonomic units (OTUs) of fungi were detected. The fungal community is dominated by ectomycorrhizal fungi belonging to the families Russulaceae, Inocybaceae, Thelephoraceae, Cortinariaceae, and Sebacinaceae – that are essential for tree growth. For fungi, nearly 36 % of OTUs and 52 % of sequences are attributed to the species level, and 1176 species were recognized, while inventories revealed 1055 species. However, only 200 species were common to both eDNA metabarcoding and inventories. Finally, communities were characterized by a high local turn-over and high beta diversity among plots. This study based on eDNA metabarcoding confirms the rich diversity of fungal communities in the Massane reserve. Estimating the reserve fungal diversity remains limited by the lack of taxonomic resolution for short barcodes, and both macroscopic and eDNA bases inventories remain complementary. Finally, the eDNA metabarcoding of soils revealed a high alpha but also beta diversity and confirms the importance of ancient forest soils and old-growth forests for the conservation of fungi, and points out the importance of ectomycorrhizal fungi in the functioning and resilience of these forests and their landscape.

HORIZONTAL TRANSFERS IN THE MASSANE FOREST: THE WELL-KEPT SECRET OF CLIMBING PLANTS.

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Horizontal transfers (HT) refer to the exchange of genetic material between divergent species through mechanisms other than reproduction. In recent years, several studies have demonstrated the existence of horizontal transfers in eukaryotes, particularly in the context of host-parasite relationships. However, our understanding of genetic material exchanges in natural ecosystems, particularly those involving wild species, and the nature of the ecological relationships that promote these exchanges, remains limited. As part of this study, we undertook a pilot study on horizontal transfers by sequencing the genomes of 17 non-model wild species from the Massane forest. Using this data, we have identified frequent transfers between lianas and trees. These observations lead us to hypothesize that these interactions promote the passage of genetic material between different species.

DIVERSITY OF EPIPHYTIC BIOFILMS PRESENT ON MASSANE TREE TRUNKS.

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Microorganisms play a key role in forest functioning. They are involved, for example, in recycling organic matter and protecting against pathogens. They colonize multiple ecological niches, particularly by developing in the form of biofilms on the surface of tree trunks, leaves, or litter. While the diversity of microorganisms colonizing forest soils has been documented, the microbiome of other ecological niches remains largely unexplored. Furthermore, recent molecular techniques for high-throughput analysis of genetic markers remain little used in these studies, even though they have revealed the existence of a very significant and unsuspected diversity of living organisms in other ecosystems, far greater than that which can be characterized by conventional culture or microscopy approaches. During the FORESTFILM project, we explored the diversity of microbial biofilms developing on living tree trunks compared to those developing on dead tree trunks by combining molecular and microscopic approaches. This work contributes to the development of protocols and approaches that will, in the long term, enable us to characterize the entire Massane microbiome and its functional role, following the example of major scientific campaigns currently exploring the microbiomes of the planet's main ecosystems.

AN OLD-GROWTH FOREST & MEN

TREE LEAVES: A TOOL FOR ANALYZING MICROPLASTICS IN THE ATMOSPHERE?

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Only a few studies analyze microplastics deposited on leaf surfaces. Still, a recent approach identified factors likely to influence plastic concentration in order to validate the use of leaves as passive samplers. In collaboration with the Massane Forest National Nature Reserve, we conducted a study to assess plastic concentrations in the atmosphere and on tree leaves collected in the city of Perpignan and in the Massane Forest, between 650 and 1,100 meters above sea level, 40 km from the city. This old forest, which has been evolving freely for over 150 years, has been extensively inventoried and is considered an open-air laboratory. The diversity of the sampling also gives us access to the variability of plastic concentrations between tree species: holm oak (*Quercus ilex*), pubescent oak (*Q. humilis*), beech (*Fagus sylvatica*), and stone pine (*Pinus pinea*). An analytical approach that is still rarely used, pyrolysis coupled with gas chromatography and tandem mass spectrometry (Py-GC-MS/MS), can be used to determine the concentrations of six plastics. The emerging trend reveals higher plastic concentrations in cities and in holm oak leaves. Analysis of atmospheric samples is still ongoing and will determine whether leaves can be used as passive samplers.

GEOCHEMICAL VARIABILITY OF ATMOSPHERIC DEPOSITS IN THE MASSANE NATIONAL NATURE RESERVE

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Since 2017, a device for collecting atmospheric deposits has been installed outside and under the forest cover at the Massane National Nature Reserve site in order to characterize the geochemistry of the deposits and quantify the fluxes of nutrients and trace metals that are potential contaminants. Similar monitoring carried out at the Cap Béar site as part of the SNO – National Observatory System MOOSE project provides insight into changes in the geochemistry of deposits over a distance of several kilometers in relation to distance from the coast and the effect of altitude. Under forest cover, the influence of excretion by tree foliage is significant for certain elements such as potassium.

ARCHEOLOGY OF A SACRED MOUNTAIN. HUMAN OCCUPATION AND MOVEMENT IN THE MEDITERRANEAN PYRENEES.

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Archaeological research conducted over the past decade in the Massane National Nature Reserve has advanced our understanding of human occupation of the mountain, providing an overview of the entire environment, from the Mediterranean Sea to the summit of the Pyrenees. This work (surveys, test pits, and excavations) has identified diachronic occupations from the Neolithic period to the present day. Located at the eastern end of the Pyrenean massif between France and Spain, this border area occupies a strategic position for traffic, trade, and cultural contact, particularly during Protohistory and Antiquity, a period when a spring sanctuary was established at the summit of the Pyrenees.