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(1) Influence of microenvironments generated by tree canopy on soil C and microbial functions.

Alessandra LAGOMARSINO[1], Roberto LAI[2,3], , Luigi LEDDA[2,3], Pier Paolo ROGGERO[2,3]

[1]*Consiglio per la Ricerca e la sperimentazione in Agricoltura - Research Centre for Agrobiology and Soil Science (CRA-ABP), Firenze, Italy*
[2]*Desertification Research Centre, University of Sassari, Sassari, Italy*
[3]*Department of Agriculture, University of Sassari, Sassari, Italy*

Microenvironments generated by tree canopy projection strongly influence soil C storage and cycling (through above- and belowground litter and rhizodeposition) and water regime (through the interception of precipitation), and generate specific microclimate conditions that may affect chemical, biochemical, and biological processes. Eight microenvironments characterized by different long-term soil management practices and different positions with respect to woody plant canopy soil vertical projections were selected in a Mediterranean agropastoral system. Four management types were considered: pasture, hay crop, grass-covered vineyard, and tilled vineyard. The aims of the study were 1) to explore the spatial variability associated to the trees for soil C storage and its components and reactivity; and 2) to assess the similarities between microenvironments in terms of microbial functional diversity. Soil organic C, microbial biomass, and respiration were measured to assess C storage and dynamics, while functional diversity was determined by means of soil enzyme activities. The results showed that the microenvironmental variation of soil organic C and functional microbial diversity generated by the tree canopies in the wooded grassland can be very relevant for an accurate assessment of soil organic C content and its dynamics. Under tree canopy in the grassland we found the highest soil organic C content, which was correlated to a high enzymatic activity. The soil microbial functional diversity increased with decreasing level of anthropic pressure in different microenvironments. According to these results, it appears relevant to consider spatial variability when assessing the C sink capacity and the C dynamics of wooded grasslands; this variability appears less important in the case of woody systems with higher level of anthropization.

(2) Spruce forest fragments versus ski runs – structural and functional diversity of soil nematode assemblages.

Alexander Mladenov, Milka Elshishka, Vlada Peneva

Institute of Biodiversity and Ecosystem Research, 2 Yurii Gagarin Street, 1113 Sofia Bulgaria

Nematodes are very diverse microscopic roundworms most numerous among all soil-dwelling animals. As a major component of all soil food webs they are involved in organic matter transformation. Ubiquitous, representing different trophic levels and life history traits, they can serve as useful indicators of soil/ecosystem conditions classified according to other ecological characteristics, such as 'life history traits'.

Diversity of nematode assemblages from *Picea abies* L. and ski runs sites in Pirin Mountain, Bansko winter resort, was assessed. The sampling plots (10x10 m², 5 soils samples) represented spruce forest fragments (7) and ski runs of different age and vegetation (6) on the northern slope of the mountain at 1800 m a.s.l. Data of soil properties and plant cover were collected for each sample and site. The average relative abundance and generic richness were higher in forest sites 418 - 771 spec./100 cm³ and 13-20 genera *vs* 2-76 spec./100 cm³ and 2-10 genera in ski run plots. Although taxonomic composition of the core genera in the two type of microhabitats was similar, their relative abundance differed. Yet, taxonomic structure was more similar between forest sites compared with ski runs caused by the more pronounced differences in terms of vegetation and soil characteristics. Furthermore, nematode functional groups distribution showed different patterns. Trophic and c-p groups structure was site-specific in ski runs, with low proportion of fungal feeders in most of the plots; the best presented group in terms of life strategy was c-p 4 followed by c-p 5 (in half of the sites only) and c-p 2 groups. In forest fragments trophic structure was dominated by predatory nematodes (16.3-54.6%) with almost equal proportions of omnivores (15.4-23%), and high percentage of bacterial or fungal feeders coupled with comparatively low participation of plant parasites; c-p 3 and c-p 4 were the best and almost equally represented groups (av. 35-36%), only in one site c-p 2 prevailed (52%). In both type of habitats plant parasitic nematodes were assigned mainly to p-p 2 and p-p 3 groups. In general maturity indices were higher again in spruce plots with a few exceptions.

In conclusion, assemblages of terrestrial nematodes of ski runs, represented by grass communities developed after cutting the spruce trees, resembled spruce forest nematode communities in generic composition but were less abundant and rather different in their functional parameters. Derived ski runs nematode assemblages can be characterised with higher percent between site dissimilarities of studied ecological parameters compared with the original forest ones. This apparently reflected the present soil conditions and vegetation characteristics following various anthropogenic impacts.

(3) Belowground biomass of young scots pine (P*inus sylvestris* l.) in forest types on dry mineral soils.

Andis Bārdulis, Mudrīte Daugaviete, Āris Jansons
Latvian State Forest Research Institute "Silava"
e-mail: andis.bardulis@silava.lv

For centuries there has been a demand for stem fraction valuation in volumetric units. That is why in forest inventory several simple and accurate methods for establishing individual tree stem volume and tree group growing stock. By knowing the wood density values it is possible to recalculate the volume estimation in the units of mass. However there is a problem with establishing non-stem fraction biomass, where this methodical indication is not suited for calculation of volume of these fractions, due to different difficulties and reasons.

The main objects of research are 13 Scots pine (*Pinus sylvestris L.*) stands (8, 12, 13, 14, 24, 45 years old) that have been established in forest land and afforested former agricultural land. The stands of *Pinus sylvestris* selected for the research are of different age, different types of forest renewal and grown in the land of different previous utilization. In total 116 sample trees were selected and their root systems were excavated.

In forest land stands of similar age there are no significant statistical differences observed between the average total biomass of sample trees in naturally forested stands and planted stands, but in the planted stands there is a tendency to produce greater amounts of root biomass. While in the afforested former agricultural land the trees of similar age make greater amount of root biomass than the trees in the forest land. The biggest fraction of biomass is coarse roots making on average 51% of the total root biomass, stump 30%, small roots 13%, and fine roots 6% of the total root biomass.

The linear regression model has been developed which includes one factorial feature (D^2H), and the root biomass is calculated as a function using the tree parameters that are easily measured. The root biomass recalculation ratios have been calculated based on the correlation between the respective fraction biomass and stem volume. Within the framework of this research, by developing the total root biomass equation, several models were tested, and the most precise was the linear regression model which includes one factorial indication (D^2H).

The information obtained as a result of regression analysis explains and describes the newly developed model. After analysing different variables, the variable indication D^2H and resulting indication were included in the regression model total root biomass. The correlation ratio in forest land is 0.98 and 0.94 in afforested agriculture land LNZ (Pearson's correlation), which indicates close linear connection between the resulting and factorial feature. The determination ratios are high in both cases, respectively $R^2 = 0.96$ and 0.89. Thus 96% and 89% of the total biomass variation can be explained by the developed linear regression model. Root biomass calculation regression equations:

sample tree root biomass in forest land ($DBH_{1.3}$ between 1.5 and 26.5 cm)
$$m = 1.270 + 107.920 \cdot D^2H ;$$

sample tree root biomass in afforested LNZ ($DBH_{1.3}$ between 3.7 and 19.8 cm)
$$m = 8.325 + 70.689 \cdot D^2H,$$

where:

m – naturally humid mass, kg;

D^2H – multiplication of squared tree $DBH_{1.3}$ (m) and the tree height (m).

Root biomass calculation regression equations were calculated for different root fraction.

(4) Tree species diversity decreases soil microbial process rates in an elevated CO_2 atmosphere.

Andrew R. Smith[a,*], Martin Lukac[b], David L. Jones[a], Douglas L. Godbold[c]

[a]School of Environment, Natural Resources and Geography, Bangor University, Gwynedd, LL57 2UW, UK [b]School of Agriculture, Policy and Development, University of Reading, Reading, Berkshire, RG6 6AR, UK. [c]Institute of Forest Ecology, Universität für Bodenkultur (BOKU), 1190 Vienna, Austria.

Tree species' identity is known to influence biogeochemical cycling, but there is a lack of understanding of how tree species richness affects ecological function and belowground interactions. Soil microbial communities have a seminal role in maintaining cycling nutrients and maintaining ecosystem function through weathering processes, organic residue mineralisation and regulating nutrient availability. Free Air Carbon dioxide Enrichment (FACE) has often been used to investigate the response of forest ecosystems to future predicted climate. To investigate the interaction between tree mixture and elevated CO_2, *Alnus glutinosa*, *Betula pendula* and *Fagus sylvatica* were planted in areas of single species and a three species polyculture in a free-air CO_2 enrichment study (BangorFACE). The trees were exposed to ambient or elevated CO_2 (580 ppm) for 4 years. Community level physiological profiles were determined from soil collected under three tree species grown in an enriched CO_2 atmosphere in monoculture and a three species polyculture. Soil was amended with sixteen different low-molecular-weight organic compounds varying molecular complexity and substrate quality. We examined the microbial uptake kinetics by collecting evolved $^{14}CO_2$ and fitting first order decay models. When species were grown under elevated CO_2 in monoculture the overall response to substrate addition was an increase in catabolic respiration. However, when the studied species were grown in polyculture and exposed to elevated CO_2 a decrease in catabolic respiration was observed relative to ambient conditions. Our results suggest that either resource limitation constrained microbial catabolic simulation in mixed species plots or species' niche differentiation reduced plant derived inputs per unit of soil and thus reduced mineralisation rates.

(5) Seedling growth differences in ecto- and endo-mycorrhizae inoculated cedar (*Cedrus libani*) in a nursery experiment conducted in inland part of Turkey.

Bulent TOPRAK*, Oktay YILDIZ*, Murat SARGINCI*
*Faculty of Forestry. DuzceUniversity. Duzce, Turkey

Data from different parts of the world suggest that mycorrhizae inoculation can significantly (up to500%) increase the seedling survival and growth rates. There are about 5.25 million ha of socially, economically and ecologically available land area for afforestations in Turkey. The significant parts of these potential afforestation areas suffer from water deficiency during vegetation season. Therefore the objective of this study is to compare the nursery performance of ecto- and endo- mycorrhizae inoculated and non-inoculated cedar seedlings' in inland part of Turkey.

A completely randomized design was used for the study. One-year-old 90 cedar seedlings grown in the nursery for afforastation stock were randomly sampled. For 30 of the seedling endo-mychorizae was inoculated using a commercial cocktail. For the next 30 of the seedling ecto-mychorizae cocktail was used. For there maining 30 seedlings no treatment was applied. For each sample, inoculation rate, shoot height, root collar diameter, root length, leaf area index, shoot dry weight, root dry weight, shoot fresh weight, root fresh weight and dickson quality index variables were measured.

Analysis of the data indicated that mycorrhizal differences were effective on morphological characteristics. There were statistically significant difference ($p \leq 005$)in the shoot height, root collar diameter, root length, leaf area index, shoot dry weight, root dry weight, shoot fresh weight, root fresh weight and shoot to root dry weight ratio and dickson quality index (P-values = 0.0148; 0.0104; 0.0434; <0.0001; 0.0010; 0,0001; 0.0022; 0.0051; 0.0488; 0.0032, respectively).

(6) Can forest management influence on the soil GHG fluxes?

Cosmin Bragă[1], Olivier Bouriaud[2], Lucian Dincă[2], Gheorghe Spârchez[1]

[1]*Faculty of Silviculture and Forest Engineering, Transilvania University of Brasov, Romania*
[2]*Forest Research and Management Institute, Romania*

The aim of the study is to evaluate the impact of forest management on the soil GHG (CO_2, CH_4, N_2O) fluxes. A set of 12 plots of monitoring was settled in a beech forest in Romania with contrasted forest management types: 5 shelter wood stands (even-aged) with varying thinning intensities, 3 selection cutting (uneven aged) stands, 1 all-aged stand (tree-level selection cutting). The site was selected for its large gradient of management types, the uniformity of the growing conditions due to uniform soil types, thus low confounding factors, time stability: treatments have been applied for over 40 years.

GHG gases were measured by chromatography. Manual chambers were used to collect samples during two successive years.

Inventories were made to analyze the stand structure and better describe the stand attributes. The results show a great contrast between treatments in terms of diameter distribution. The high variability in density or volume makes a contrast in GHG fluxes more probable. However extreme situations were excluded and the values obtained are typical for the stands sampled, the representatively of the plots selected is ensured.

Forest management had no effect on the soil GHG fluxes. Even-aged and uneven aged stands proved to have similar ranges and very synchronic fluctuations of GHG soils fluxes.

Climatic conditions and difficulties related to the GC auto-calibration limited the results. Further investigations will be necessary such as a longer-term monitoring.

(7) Forest litter biodiversity of decomposition hotspots: fungal community composition in the forest floor at a centimetre scale.

Diana Gálová, Petr Baldrian

Laboratory of Environmental Microbiology, Institute of Microbiology of the ASCR, Videnska 1083, 14220 Praha 4, Czech Republic

In forest ecosystems, decomposition of litter is limiting step of carbon cycling and an essential process leading towards soil formation. This process is carried out mainly by fungi, which possess effective degradative enzymes. Forest soils show considerable spatial heterogeneity of enzyme distribution as well as fungal community composition. Diversity of litter decomposers was described at different levels, from metres to kilometres, and in this work we asked if this spatial heterogeneity can be seen even at a scale of an individual leaf. To link fungal community composition to decomposer activity, we analysed fungal DNA in decomposition hotspots with high exocellulase activity and in nonactive patches. Litter was collected at different stages of decay in a *Quercus petraea* forest - leaves before litterfall and after 2, 10 and 22 months of decomposition. We measured the activity of exocellulase over the leaf surface and areas with the high and low enzyme activity were identified in each leaf. These areas of approx. 1 cm^2 were cut out, DNA was extracted and fungal community was analysed using ITS2 sequencing. Exocellulase activity varied considerably over the surface of a leaf (up to 20-fold) and the highest activities were detected after 10 and 22 months of decomposition. In one spot we found in average 42 genera, ranging from 20 to 68, what gives evidence for substantial diversity even at such small area as 1cm^2. Community of active and nonactive areas within a single leaf were different, but not in a consistent way. There was, however, a clear shift in community composition across litter of different stages of decay. Early stages were dominated by endophytes, saprotrophic basidiomycetes increased with time. Our results show that fungal communities are spatially heterogeneous even at the level of individual leaves.

(8) Microbial and functional diversity of the endophytic community in pine trees from affected areas with pine wilt disease.

Diogo Neves Proença[1], Romeu Francisco[1] and Paula V. Morais[1,2]

[1]IMAR-CMA, University of Coimbra, Coimbra, Portugal
[2]Department of Life Sciences, FCTUC, University of Coimbra, Coimbra, Portugal

e-mail: diogo.proenca@gmail.com

Abstract

Pine wilt disease (PWD), caused by the pinewood nematode *Bursaphelenchus xylophilus*, is one of the most devastating forest diseases in the world and has created a tremendous economic, social and environmental impact. This study aimed to assess the endophytic microbial (bacterial and archaeal) community of PWD-susceptible *P. pinaster* by molecular and cultivation methods, and to assess the diversity of functional genes involved in nitrogen fixation (*nifH* and *nifJ* genes) and nitric oxide production (*nirK* and *nirS* genes). Members of the classes *Acidobacteria*, *Actinobacteria*, *Alphaproteobacteria*, *Bacilli*, *Betaproteobacteria*, *Flavobacteria*, *Gammaproteobacteria* and *Sphingobacteriia* were identified as endophytes by both methods and *Bacteroidia*, *Deinococci*, *Fusobacteria, Spirochaetes, Planctomycetaceae* and *Verrumicrobiae* were only detected by DGGE. Archaea were found as part of the endophytic community of pine trees, including members of the phyla *Euryarchaeota, Thaumarchaeota* and *Crenarchaeota*. The population of nitrogen fixing bacteria indicated by the *nifH* and *nifJ* genes was diverse within *Gamma-* and *Alphaproteobacteria*. The prevalence of *nir* genes appeared to be related with the sampling area and the structural diversity of the endophytic community. This study contributes to increase the knowledge about the endophytic microbial community of pine trees consisting of Bacteria and Archaea and whether they have a role for the plant.

(9) Soil chemical and microbiological properties in relation to harvest sequence in mountain beech and fir forests on calcareous bedrock

Emira Hukić[1], Čustović H.[2], Ballian D.[1]

1 Faculty of Forestry University of Sarajevo, Zagrebačka 20, 71 000 Sarajevo; Bosnia and Herzegovina (e.hukic@gmail.com)
2. Faculty of Agriculture and Food Science, University of Sarajevo, Zmaja od Bosna 8, 71 000 Sarajevo; Bosnia and Herzegovina

Abstract

Mixed beech and fir forests on calcareous bedrock are among the most abundant forest types in Bosnia and Herzegovina. Inventory data implicate that these forests account for even 50 % of all high forests and they are considered as the most important wood recourses for the economy of forest enterprises in the country. Apart from their economic importance, they also provide a series of necessary ecosystem services. Since soils in these forest ecosystems are attributed to a very slow development, they represent a very valuable component, which therefore needs to be utilized carefully. Among all forest management activities, forest harvest may be considered as the major factor influencing site disturbance and controlling soil processes. Therefore, the main aim of this study is to explore the effects of forest harvest on soil chemical and microbiological properties. In order to investigate the effects of forest harvest related to forest management practice, this study focuses firstly on site measurements and collecting the data of microclimate parameters, forest canopy and forest floral elements. Site factors will offer a strong potential for interpreting influences of spatial and temporal changes in forest structure on soil organic matter and soil microbial properties.

Key words: beech and fir forests, site factors, forest harvest, soil, microorganism

(10)   Provision of soil and woodland ecosystem services on reclaimed land through earthworm activity

Francis E. Ashwood[1*]**,** Butt, K. R. [1], Doick, K. J. [2], and Vanguelova, E. [2]

[1]Earthworm Research Group, University of Central Lancashire, Preston, PR1 2HE, UK

[2]Forest Research, Alice Holt Lodge, Farnham, Surrey, GU10 4LH, UK

[1*]E-mail contact: feashwood@uclan.ac.uk

The use of a suitable soil resource is essential to provide the necessary chemical, physical and biological conditions for sustainable woodland establishment on reclaimed land. There is an increasing industrial and scientific interest in improving the soil resource for reclamation projects, particularly through the addition of organic matter from waste streams. Additionally, whilst it is recognised that earthworms play a crucial role in soil development and the cycling of essential plant nutrients and organic carbon, much is still unknown about their potential for improving the delivery of ecosystem services by soil and trees on reclaimed land.

This work describes the early stages of a PhD research project, and presents the methodologies and preliminary results of experiments carried out to date. These include a field experiment at a reclaimed woodland site, a pot-based field experiment, and a lab-based microcosm experiment. This research will contribute knowledge regarding the effects on tree growth, soil biodiversity, and soil quality on reclaimed land through the activity of earthworms and organic waste addition; and improve our understanding of the dynamics of naturally and artificially colonised earthworm populations in brownfield land reclaimed to woodland.

(11)   Fine root traits, belowground interactions and competition effects of Beech and Ash

Friderike Beyer

Email: friderike.beyer@slu.se

European beech (*Fagus sylvatica* L.) and European ash (*Fraxinus excelsior* L.) are common tree species in Central European forests and of high ecologic as well as economic value. However, knowledge about the structure and function of the ecologically important fine root system of beech and ash and its impact on rhizosphere processes is scarce. Moreover, little is known about the direct intra- and interspecific belowground competition effects of these two species. The main objective of the study was to disentangle species-specific effects from competition/biodiversity effects on rhizosphere and fine root properties.

In a competition experiment with saplings of beech and ash grown in different rhizoboxtreatments (monoculture, mixture or single plant) we investigated morphological, C/N and δ13C responses in the fine root system employing a root order-related analysis. We observed large differences in various root traits between the root order classes 1 to 4, which underscores the ecological significance of the position of roots in the root system, e.g. 1st order roots, i.e. root tips, had significantly higher specific root areas and contributed to 65-70% to the total length of the analysed root segments. While the species specific fine root characteristics of beech and ash were obvious, no major root morphological or chemical (nitrogen concentration, C/N ratio) alterations in response to competition were found. This partly contradicts observations in mature stands, where fine roots of beech were shown to act very plastic in changing their specific root length in a competitive environment. Thus, adaptive root responses to competition may not be a universal phenomenon and likely vary with site conditions, species and plant age. In contrast to the fairly unaffected root morphological and chemical traits, fine root survival, which was analysed by sequential digital imaging of root growth through a root window, showed significant differences between competition treatments and species. Competition with conspecific or allospecific roots altered root longevity in both directions, either toward a shorter lifespan or greater longevity. Mean root lifespan differed significantly among species with higher fine root longevity in ash and also depended on competition treatment. Fine root mortality increased in beech roots grown in mixture with ash and in beech monoculture compared to beech plants grown in isolation. Ash fine roots apparently profited from the presence of beech roots, while beech root growth and survival were negatively affected by ash. These results indicate size-asymmetric belowground competition. Thus, competition represents an important force influencing the fine root lifespan of beech and ash saplings.

(12)   A new SCOPES project looking into the biodiversity of arbuscular mycorrhizal fungi in selected biodiversity hot spots of the Balkan Peninsula

Irena Maček [1, 2, *], Žaklina S. Marjanović [3], Fritz Oehl [4]

[1] Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[2] Faculty of Mathematics, Natural Sciences and Information Technologies, University of Primorska, Glagoljaška 8, 6000 Koper, Slovenia
[3] Institute for Multidisciplinary Research, University of Belgrade, Kneza Višeslava 1a, 11 000 Beograd, Serbia
[4] Agroscope, Federal Research Institute of Sustainability Sciences, Plant-Soil Interactions, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland

* Correspondence: irena.macek@bf.uni-lj.si (I. Maček)

Arbuscular mycorrhizal (AM) fungi are plant root endosymbionts and important, ubiquitous organisms in soils belonging to the phylum Glomeromycota. They improve plant mineral nutrition, maintain plant health, increase pathogen resistance and water availability, and are involved in several other important ecosystems services (e.g. carbon and nutrient cycling, soil aggregation). Despite the fact that they have been proven as an important component of forest ecosystems, research into their communities in different forest types is still very scarce, especially within Eastern Europe, including the Balkan Peninsula. Alongside Iberia, this area harbours the richest flora in Europe, possessing not only the largest number of species, but also hosting most endemics. The Balkan Peninsula, however, represents an unstudied region regarding research on any aspect of AM fungal ecology or biodiversity. Within a newly funded SCOPES (Scientific Co-operation between Eastern Europe and Switzerland) project, biodiversity and community ecology of AM fungi will be studied in selected biodiversity hot spots of the Balkan Peninsula (mainly Slovenia and Serbia) based on concomitant morphological and phylogenetic analyses. Simultaneously, high resolution amplicon sequencing of AM fungal DNA from plant roots and morphologically and molecular-based surveys of AM fungal spores from soil and trap cultures will be used in order to study the changes in AM fungal community composition across a range of different ecosystems (e.g. different types of pristine forests, secondary succession areas). We will test if the observed patterns in AM fungal communities are seasonally stable, and try to determine the main predictors of the spatial and temporal AM fungal community compositional shifts. Research into this valuable genetic pool is not only important from a biodiversity view point, but also represents a resource for site-directed reclamation of disturbed soils (ecosystems), sustainable forestry, nature conservation, and agriculture that has not been used as such in Balkans so far.

(13)   NGS approaches for soil microbial diversity and ecology studies in nematode-plant interactions.

Isabella Pentimone[*], Mariantonietta Colagiero, Laura C. Rosso and Aurelio Ciancio

Istituto per la Protezione delle Piante, CNR, Via Amendola 122/D, 70126 Bari, Italy. E-mail:

[*] isapentimone@yahoo.it - PostDoc IPSP and Presenting Author

The study of soil microbial diversity involves many cryptic species. Only less than 1% of bacterial species are in fact culturable *in vitro*. Concurrently, many services provided by known species are yet unexplored. Next generation sequencing (NGS) approaches may allow the study of a large fraction of soil species and services, through their DNA/RNA sequences. In a first NGS-based study, we tested *in vitro* the effect of the endophytic fungus *Pochonia chlamydosporia* (*Pc*) on tomato gene expression. Endophytic fungi are key members of soil microbial communities and can regulate many pests. *Pc* is a root knot nematode (RKN) biocontrol agent acting endophytically in roots of annual and perennial crops. We sequenced the fungus transcriptome in tomato roots from plants kept in flasks with sterile vermiculite for three weeks, with or without (control) the fungus. RNAs of roots and *Pc* were extracted and sequenced with an Illumina HiSeq. Based on $46\cdot10^6$ high quality reads mapped on the SL2.40.15 tomato genome, we identified differentially regulated genes in colonized roots. 9112 were mainly related to transport, secondary metabolism and stress response, with significant expression of 213 transcripts linked to plant defense, including disease resistance gene families members (NBS-LRR, CC and others). The *Pc*-induced genes included *Mi* (for resistance to RKN), NAC and WRKY transcription factors, and proteinase inhibitors involved in resistance to stress. Main categories associated to *Pc* genes expressed in roots were protein translation, signal transduction, regulation and transport. Induced resistance is fundamental to sustain plant protection, and NGS data allowed a deeper insight on the *Pc* ecology and role as a beneficial rhizosphere organism.

In a second study, to evaluate factors affecting soil bacterial diversity, a metagenomic NGS protocol was implemented to identify ecological links among RKN, soil bacteria and chemical treatments. A RKN-free organic soil planted with tomato was studied. Treatments included RKN inoculated plants, with or without a nematicidal (fenamiphos), and treated/untreated RKN-free soils as controls. Samples were collected from pots at three time intervalss. The total RNAs, extracted and sequenced (Illumina MiSeq System) using the V4 - 16S rRNA region, yielded > 6.65 · 10^6 sequences, allowing identification of > 97% of taxa. Dominant groups were Proteobacteria, Gemmatimonadetes, Actinobacteria, Bacteroides with varying frequencies, per treatment and time. Less Proteobacteria and more Actinobacteria and Gemmatimonadetes were found in controls during the time course. RKN increased by 24% the Proteobacteria, decreasing Actinobacteria, Bacteroides, Gemmatimonadetes by 13%, 2% and 5%, respectively. Similar behavior was found in inoculated and fenamiphos treated samples. Fenamifos increased Proteobacteria by < 14% when compared to samples inoculated with RKN, reducing Actinobacteria (7%) and Bacteroides (3%). NGS data at finer taxonomic scales showed groups or genera directly affected by nematodes and chemical treatments. Studies are in progress to adapt metagenomic and single species NGS approaches to gene expression studies in Mediterranean tree crops (olive, grapevine), to detect microbial community services related to soil pests.

Keywords: soil bacteria, fungi, Mediterranean tree crops, metagenome, transcriptome

(14) Environmental drivers of ectomycorrhizal communities in Europe's temperate oak forests.

Laura M. Suz1,2, Nadia Barsoum3, Sue Benham3, Hans Peter Dietrich4, Karl Dieter Fetzer5, Richards Fischer6, Paloma García7, Joachim Gehrman8, Ferdinand Kristöfel9, Miklós Manninger10, Stefan Neagu11, Manuel Nicolas12, Jan Oldenburger13, Stephan Raspe4, Gerardo Sánchez7, Hans Werner Schröck14, Andrea Schubert4, Kris Verheyen15, Arne Verstraeten16 and Martin I. Bidartondo1,2

Ectomycorrhizal fungi are major ecological players in temperate forests but they are rarely used in measures of forest condition because large-scale, high-resolution, standardized and replicated belowground data is scarce. We carried out an analysis of ectomycorrhizas at 22 intensively-monitored long-term oak plots, across nine European countries, covering complex natural and anthropogenic environmental gradients. We found that at large scales mycorrhizal richness and evenness declined with decreasing soil pH and root density, and with increasing atmospheric nitrogen deposition. Shifts in mycorrhizas with different functional traits were detected; mycorrhizas with structures specialized for long-distance transport related differently to most environmental variables than those without. The dominant oak-specialist Lactarius quietus, with limited soil exploration, responds positively to increasing N inputs and decreasing pH. In contrast, Tricholoma, Cortinarius and Piloderma species, with medium-distance soil exploration, show a consistently negative response. We also determined N critical loads for moderate (9.5 – 13.5 kg N ha-1 yr-1) and drastic (17 kg N ha-1 yr-1) changes in belowground mycorrhizal root communities in temperate oak forests. Overall, we generated the first baseline data for ectomycorrhizal fungi in the oak forests sampled, identified nitrogen pollution as one of their major drivers at large scales, and revealed fungi that individually and/or in combination with others can be used as belowground indicators of environmental characteristics.

(15) Forest management adaptation to climate change – A Cornelian dilemma between drought resistance and soil macro-detritivore functional diversity

Ludovic Henneron[a], Michaël Aubert[a], Fabrice Bureau[a], Yann Dumas[b], François Ningre[c], Sandrine Perret[b], Claudine Richter[d], Philippe Balandier[b] and Matthieu Chauvat[a*]

a Laboratoire d'Ecologie, EA 1293 ECODIV, Fédération de Recherche SCALE, Bâtiment IRESE A, UFR Sciences et Techniques, Université de Rouen, F-76821 Mont Saint Aignan, France
b IRSTEA, Unit Research on Forest Ecosystems (EFNO), Domaine des Barres, F-45290 Nogent-sur-Vernisson, France
c INRA, UMR 1092, LERFoB, Centre INRA de Nancy, 54280 Champenoux, France
d ONF, Research & Development department, Boulevard de Constance, 77300 Fontainebleau, France

Global warming induces new constraints on forest ecosystems and requires forest management adaptation. The reduction of stand density is currently debated in France as a potential tool to face increasing summer drought risk by improving forest resistance to climate change-induced tree mortality. However, few studies have yet assessed the potential impact of this management change on soil biodiversity.

We conducted a large-scale assessment of soil macro-detritivore assemblage response to stand density experimental manipulation. A total of 33 stands were studied covering a wide gradient of stand density, *i.e.* stand basal area from 2.5 to 43.7 m2.ha-1, stand age, *i.e.* 18 to 171-year-old, and pedoclimatic context conditions.

We observed contrasted responses according to both taxonomic and functional group considered. Exploratory analysis highlight that those changes were mainly related to understorey vegetation, microclimatic and soil pH condition alterations. Soil macro-detritivore assemblages response to stand density manipulation was consistent over the stand age gradient.

Among the litter-dwelling macro-detritivores, millipede abundance and diversity decreased with stand density reduction while woodlice and epigeic earthworms were unaffected. Further, a shift in the composition of soil-dwelling earthworm assemblage was observed. Endogeic earthworm abundance and diversity showed a strong increase with stand density reduction whereas anecic earthworm abundance tends to decrease. The estimated species richness of anecic earthworms followed a significant humpbacked relationship with highest values at intermediate stand density.

Our study provides evidences that reducing stand density will have substantial impacts on soil macro-detritivore assemblages, especially in mull stands. Functional consequences of those alterations are discussed in light of functional redundancy and insurance hypothesis theories. Managing stand density of oak forest at intermediate level, *i.e.* 25 m2.ha-1, appears as the best way to optimize the trade-off between improving forest resistance to climate change and ensuring the conservation of functional diversity to preserve forest ecosystem functioning and stability.

Keywords: Aboveground–belowground interactions; Soil biodiversity; Soil macrofauna; Earthworms; Diplopoda; Isopoda; Biodiversity conservation; Soil functioning; Land management; Global change.

(16)   Understanding linkages between soil belowground microbial diversity and plant roots influencing soil aggregate stability

*Luis Merino-Martín*1,2, *Robert Griffiths2, Yves Le Bissonnais3, Alexia Stokes1*
1 INRA, UMR AMAP, Montpellier, F-34000, Montpellier, France.
2 Centre for Ecology & Hydrology, Maclean Building, Benson Lane, Crowmarsh Gifford, Wallingford, Oxfordshire, OX10 8BB, UK.
3 INRA, UMR1221 LISAH, 2 place Viala, F-34060, Montpellier, France.

Careful management of land can prevent the detrimental consequences of farming on soils, but both fundamental and applied research are required to determine how best to manage depending on land use, climate and topography. The present research project investigates the ecosystem service of soil fixation provided by different crop / forest systems examining root traits and soil microbial communities. The specific aim of this research is to determine the function of the root systems and the soil microbial communities in relation to aggregation properties of soils. A combination of a field study and a laboratory inoculation experiment will be used in this research.

The philosophy of the field study is not to compare results from the different field sites, but to identify similarities depending on climate, soils and land uses. Field sites lie in hilly or erodible environments and represent natural forest, agroforest, arable and pasture fields in subalpine, temperate and mediterranean regions. Soil samples were collected on the different sites and aggregate stability, root traits, soil microbial communities, soil texture and soil chemical properties were studied. Bacterial and fungal community biodiversity will be assessed using Terminal Restriction Fragment Polymorphism analyses (TRFLP) coupled with high throughput sequencing of 16S rRNA genes (bacteria) or the ITS region (fungi) to identify responsive taxa. The main root traits examined will be density and biomass. Aggregate stability is studied using a standard method (ISO/CD 10930).

The laboratory inoculation experiment will be setup in the next months and results are not obtained yet. The objective of this experiment is to test if soil microbial composition mediates the effects of roots and land use on soil aggregate stability. Reciprocal inoculations of microbial communities crossed with root systems (tap vs fibrous) will be compared in their influence on the aggregation properties, root exudates and bio-films production.

The research project is still in the initial stage, so objectives, methodologies and some of the aggregate stability preliminary findings will be presented and discussed.

(17)   North American ectomycorrhizal partners of Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) in invasion range - fare dodgers or partners in good deal?

Marcin Pietras

Institute of Dendrology Polish Academy of Science, Parkowa Str.5, 62-035 Kórnik, Poland
mpietras@man.poznan.pl

The history of organisms introduction have begun about 500 years ago, after America discover. Nowadays introductions and spreading of foreign and invasive organisms is one of the most important problems in nature conservation. One of the most planted tree species in the world is Douglas fir (*Pseudotsuga menziesii*). This tree occurs naturally in North America, but because of its fast growth and good wood properties it has been cultivated outside of its native range, in Europe and South America for almost 200 years. In France and Germany, Douglas fir has become most important foreign tree species for forestry. In Poland Douglas fir was successfully introduced in 19th century but have not got significant position for timber production. Currently, in total almost 5,000 ha of Douglas fir forests is growing on areas managed by Polish State Forests National Forest Holding. In natural range Douglas fir is capable of forming mycorrhizae with as many as 2000 species of fungi. As ectomycorrhizal (ECM) symbiosis is a condition of good growth and survival of many important forest trees species, it is possible to assume that the success of tree establishment beyond of their natural distributional range, involves, among others, the availability of functional compatible symbiotic partners that may be furthermore implicated in determination of persistence of trees. Thus main objectives of this study is 1) to describe ECM fungal composition of Douglas fir outside natural range and 2) to assess potential occurrence of foreign ECM fungi assembled with *Pseudotsuga menziesii* in Poland.

Root material have been sampled in the Arboretum Kórnickie and in tree oldest (162-172 years) Douglas fir stands in Poland. From each study field root samples has been taken in June and November 2013. Additionally eight approximately 20-years old plantation of Douglas fir were studied in late autumn 2013. Ectomycorrhizas naturally colonizing Douglas firs roots was described using standard morphological and molecular approach. Molecular identification of ectomycorrhizas has been performed based on PCR and sequencing of the ITS of rDNA. Altogether 70 morphotypes, representing 41 ECM taxa, has been distinguished. The species richness ranged from 4 and 12 species per site. The most frequent taxa were: *Cenoccocum geophilum*, *Tylopilus felleus*, *Lactarius rufus* and members of thelephoroid fungi. In case of Arboretum Kórnickie as well as old forests any foreign ECM taxa has been found. In three out of eight 20-years old plantations mycorrhizas of North American species, *Suillus lakei*, were identified. Ectomycorrhizal assemblages of Douglas fir old forests, as well as plantations are characterized by high number of rare and endangered fungal species e.g. *Russula atrorubens*, *R. pectinata*, *Cortinarius croceus* or *Tomentella stuposa*. In one of plantation *Tomentella alni* has been recorded for the first time in Poland. Presented results show, that even if alien fungi has been introduced with Douglas fir to novel range, they should be regarded as a "fare dodgers", because Douglas fir is able to assembly others, native ECM fungi. This study is also a vote pro Douglas fir in Polish forests.

(18)   Ectomycorrhizal fungal community in mature poplar plantation

Marina Katanić[1], Saša Orlović[1], Tine Grebenc[2], Branislav Kovačević[1],
Milan Matavuly[3], Hojka Kraigher[2]

[1] University of Novi Sad,Institute of Lowland Forestry and Environment, Antona Čehova 13, 21000 Novi Sad, Serbia, marinakatanic44@gmail.com
[2] Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana, Slovenia
[3] University of Novi Sad, Faculty of Sciences, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

Mycorrhizal fungi are important part of belowground biodiversity with considerable influence on functioning of forest ecosystems. The aim of this study was to analyse community of ectomycorrhizal fungi in white poplar's plantation and to determine the influence of season on it. Identification of the fungal partner in ectomycorrhiza was performed by combining morphological and anatomical characterization of ectomycorrhizae with molecular identification approach, based on sequencing of the ITS rDNA region. During four seasons 30 ectomycorrhizal types were recorded on investigated site.The highest number of ectomycorrhizal types was recorded in the winter (22), then in the spring (19), while the lowest number was found in the autumn and the summer (17). Most types were identified only with molecular methods, since morphological and anatomical descriptions of ectomycorrhyza of these fungi had not existed in the literature. *Genabea fragilis, Hebeloma vaccinum, Hymenogaster olivaceus, Inocybe cincinnata, Inocybe griseovelata, Inocybe obsoleta, Inocybe squamata, Inocybe umbrinella, Peziza cf. succosa, Peziza depressa, Scleroderma bovista, Sebacina incrustans, Tuber maculatum* and *Tuber rufum* were identified to the species level; *Entoloma* sp., *Geopora sp., Inocybe* sp. 1, *Inocybe* sp. 2, *Peziza* sp., *Tarzetta* sp*., Tomentella* sp. 1, *Tomentella* sp. 2, *Tomentella* sp. 3, *Tomentella* sp. 4, *Tomentella* sp. 5, *Tomentella* sp. 6 and *Wilcoxina* sp. to the genus level; *Cortinariaceae* sp. and *Thelephoraceae* sp. to the family and one type remain unknown. Species richness index was the highest in the winter and the lowest in the summer. On the other hand, Shannon-Weaver index had the highest value in the spring and the lowest in the summer. However, no statistically significant differences were found in the diversity indices between seasons. During all seasons *Geopora* sp., *Inocybe cincinnata, Inocybe griseovelata, Inocybe* sp. 2, *Tomentella* sp. 2, *Tomentella* sp. 6, *Tomentella* sp. 3, *Tuber rufum* and *Tuber maculatum* were present. Basidiomycota group was more dominant and abundant in comparison to Ascomycota during investigated seasons. Short distance exploration type dominated ectomycorrhizal community throughout the studied year.

Katanić M (2014). Diversity of mycorrhizal fungi of poplars (*Populus* spp.) (in Serbian), PhD thesis, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia, pp. 194

(19) Linking belowground biodiversity and plants development in European White Elm plantation at the wetland

Marina Nonić, Jovana Devetaković, Mirjana Šijačić-Nikolić, Vladan Ivetić

Nonić Marina, Teaching Assistant and PhD student, University of Belgrade - Faculty of Forestry, Belgrade, Serbia E-mail: marina.nonic@sfb.bg.ac.rs (corresponding author)

Devetaković Jovana, Research Assistant and PhD student, University of Belgrade - Faculty of Forestry, Belgrade, Serbia E-mail: jovana.devetakovic@sfb.bg.ac.rs

Prof. Šijačić-Nikolić Mirjana, PhD, University of Belgrade - Faculty of Forestry, Belgrade, Serbia E-mail: mirjana.sijacic-nikolic@sfb.bg.ac.rs

Doc. Ivetić Vladan, PhD, University of Belgrade - Faculty of Forestry, Belgrade, Serbia E-mail: vladan.ivetic@sfb.bg.ac.rs

Wetlands are the transitional area between aquatic and terrestrial ecosystems, popularly known as "*biological supermarkets*"[1], because of rich aboveground and belowground biodiversity and interwoven food chains. The Great War Island is the wetland that has been created as an alluvial-accumulative and sedimentary formation, due to stopping of sandy sediments at the confluence of the Danube and Sava rivers. It is the protected natural area, located in the city of Belgrade (Serbia), which includes the presence of different types of flora and fauna, dependent to a greater or lesser degree of water amount. The rich vegetation consists of swamp and aquatic communities, as well as the different communities of alluvial forests.

According to the IUCN (*International Union for Conservation of Nature*) categorization, European White Elm (*Ulmus effusa* Willd.) belongs to the category of rare and endangered species in Serbia. The disappearance of wetland habitats, where the White Elm is represented, is the primary threat to the survival of populations and their genetic diversity, which leads to the ecological instability of this species. Through detailed terrain recognition, a total of 56 European White Elm trees were registered in three spatially isolated sub-populations, which represent the only remaining parts of former natural populations in the Great War Island. This fact indicates the importance of genetic conservation of European White Elm gene pool in this protected area.

The assessment of production and adaptive potentials of different half-sib lines represents a basis for seed collection and seedling production, aimed at spreading the population of endangered species and preservation of genetic variability. Certainly, belowground-aboveground interactions can alter plant development and productivity.

The European White Elm plantation was established at the Great War Island with 14 half-sib lines, originating from the natural sub-populations at this area. The percentage of seedlings emergence and survival was monitored during the first year after their transplanting to the permanent place. The analysis of the development of White Elm seedlings is a subject of continuous monitoring, in order to determine the linking belowground biodiversity and plants development in this plantation.

Keywords: belowground biodiversity, European White Elm, plantation, wetland

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(20) Changes in microbial community structure and functioning of a degraded soil due to the use of anaerobic digestate derived composts and rosemary plants

Martina Di Lenola[1*], Paola Grenni[1], Anna Barra Caracciolo[1]

[1] Water Research Institute, National Research Council of Italy, Via Salaria km 29,300 Monterotondo (Rome) Italy - *dilenola@irsa.cnr.it, PhD Student and Presenting Author

Since most of the soil processes are microbial mediated, soil microorganisms are central to the soil ecological functioning, providing several regulation ecosystem services. Microbial activity and soil fertility are closely related and the soil microorganisms adapts quickly to environmental constrains by adjusting their biomass, activity rates and community composition. Microbial structure and its biochemical properties can represent sensitive indicators of soil quality.

We studied the changes in the structure and functioning of the microbial community in a degraded agricultural soil after the incorporation of two composts, obtained from cattle manure or pig slurry anaerobic digestate, and the use of rosemary plants for restoring soil quality.

The composts were applied at low or high doses on soil samples (30 or 60 t ha^{-1}, respectively), which were maintained in microcosms for 6 months. Some soil microcosms were treated with inorganic fertiliser and other non-treated soils were used as microbiological controls. Rosemary plants, used both for their ability to grow in semi-arid regions and for the capacity of their root system to protect soil from erosion, were planted in half of the entire microcosm set up. At different times (0-180 days) microbial abundance, cell viability and dehydrogenase activity were measured in the different experimental treatments. Total and water-soluble soil organic carbon and nitrogen contents were assessed at 0 and 180 days. With an increase in carbon and nitrogen soil content, an increase in microbial abundance was also observed in the presence of both composts. However, microbial activity was significantly influenced by rosemary presence, without considering the allochthonous carbon and nitrogen input. Moreover, microbial community structure and diversity was also assessed by Fluorescence *In Situ* Hybridization in the different treatments. The highest values for microbial community biodiversity were found in the co-presence of the rosemary plant and at low concentration of both composts. The overall results suggest that the use of composts together with plant species suited to Mediterranean areas seems to be an appropriate strategy for restoring soil quality and the ecosystem services provided by microorganisms.

Keywords: microbial activity and diversity, soil bacteria, rhizosphere, degraded soil, organic amendments, compost

(21)　Does different management after windthrow influence belowground diversity of ectomycorrhizal fungi?

Martina Vašutová[1,2], Magda Edwards[1], Pavel Cudlín[1]

[1]Department of Landscape Carbon Deposition, Global Change Research Centre, Academy of Sciences of the Czech Republic, Na Sádkách 7, 37005 České Budějovice, Czech Republic; [2]Faculty of Forestry and Wood Technology, Mendel University in Brno, Zemědělská 1, 61300 Brno, Czech Republic

A windthrow caused by bora wind destroyed 12 000 hectares of forest (*Lariceto-Piceetum*) in Tatra National Park (Slovak Republik) in 2004. After a complicated public discussion and a wildfire, the majority of the area was cleared and a small part was left uncleared for natural development. One of the arguments for keeping destroyed forests uncleared is a maintenance of the diversity of ectomycorrhizal (ECM) fungi.

To answer the question about an influence of management on ECM species diversity we selected 16-17 spruce seedlings aged 4-9 years in the area 200 x 200 m per treatment (cleared site, uncleared site, fire site, mature forest). Ectomycorrhizas of three roots per seedling (from bottom, middle and upper parts) were divided into morphotypes and identified based on ITS sequence similarity. Fungal fruitbody surveys were done in each treatment in September 2013 and 2014 (plot 50 x 50 m).

In total 31 morphotypes were detected, 25 identified into species/genus level. Approximately 4-5 morphotypes were found per seedling, 15-18 per treatment. Occurrence of morphotypes on root systems was significantly affected by different management (explained variability 17%, CCA analysis, Canoco). Surprisingly the most similar sites according ECM species composition of seedlings were the mature forest and the cleared site. Most frequent species were *Piloderma sphaerosporum* and *Tylospora fibrilosa* in the mature forest, *P. sphaerosporum, T. asterophora, Xerocomus badius* in the cleared site, *Thelephora* sp. and *T. asterophora* in the fire site, *T. fibrillosa* and *Piloderma* sp. in the uncleared site. Based on fruitbody surveys, 36 species of spruce ECM symbionts were found in the mature forest, 9 species in the uncleared site, 4 species in the cleared site, and 2 species in the fire site.

Possible explanations for ECM community pattern on seedlings are i) totally changed conditions in the fire site (reduction of ECM propagules in soil) and the uncleared site (wood as a source of humidity and lower soil temperature) compared to the mature forest; ii) the closest distance between the mature forest and the cleared site.

Due to current lack of knowledge on ecology of most ECM species, we cannot predict consequences of different ECM species composition and a strong reduction of fruitbody formation.

(22) Effects of tree species change and clear-cut forestry on soil microbial diversity

Nahia Gartzia-Bengoetxea.1, Arias-González, A. 1, Kandeler, E. 2, Martínez de Arano, I. 1*
1 NEIKER-Tecnalia, Forestry Unit, 812 Bizkaia Science and Technology Park, E-48160 Derio, Spain
2 Institute of Soil Science and Land Evaluation, Soil Biology Section, University of Hohenheim, Emil Wolff-Str. 27, D-70599 Stuttgart, Germany
* Present address; EFIMED Regional Office, Sant Pau Art Nouveau Site, Sant Leopold Pavilion, St. Antoni M. Claret, 167, E-08025 Barcelona, Spain

Increasing population growth and demand for wood and fibres in addition to the increasing concurrence in global markets is putting greater pressure on forest ecosystems. This is usually accompanied by forest management intensification using rapidly growing exotic species and mechanization in harvesting and logging operations. Some of these forestry management activities can exacerbate soil degradation processes such as loss of soil biodiversity. The present study evaluated the changes in soil biodiversity under different types of management, involving: (i) species change and (ii) heavily mechanized forest operations. Five adjacent forest stands were selected: (i) Two unmanaged and seminatural forests (*Quercus robur*, *Fagus sylvatica*) and one (40-yearold) non mechanized *Pinus radiata* plantation, to investigate the effect of species change; (ii) two adjacent clear-cut sites (3- and 16-years- old), to evaluate the effect of mechanized forest operations 3 and 16 years after disturbance. The mechanized forest operations in the two clear-cut sites involved clearcutting with chainsaw and limbing, then tree length skidding to a landing area or forest road and finally site preparation prior to planting by blading and down-slope ripping. In each stand, three randomly selected plots (10 m x10 m) were established, and ten soil samples were systematically collected within each plot by the core method (core diameter 5 cm). The samples were divided into 0-5 cm and 5-15 cm layers and combined to provide one composite sample per plot and layer. The structural diversity of microbial community was determined by phospholipid fatty acid (PLFA) analysis and the diversity of PLFAs was calculated with the Shannon index *H*. Functional diversity by a fluorimetric MUB-based enzyme assay suitable for the study of small-scale distribution of extracellular hydrolytic enzymes of fungi and bacteria.

The Shannon diversity index was not able to detect any significant difference between tree species or intensity of forest operations. However, one of the principal "player" in the soil microbial community are fungi, and the fungal biomarker (18:2ω6) was significantly higher in mature pine stand than in oak and beech stands, presumably because fungi are more efficient than bacteria at decomposing pine litter. Although we observed slight differences in functional diversity, mature stands did not differ significantly in enzyme activities.

Mechanized forest operations resulted in a shift in bacterial community on the basis of PLFA biomarkers . There was an increase in abundance of Gram-positive bacterial PLFA with a concomitant decrease in Gram-negative bacterial PLFA indicating stress conditions induced by harvesting and mechanical site preparation in microbial communities. Furthermore, fungal biomass was significantly lower in recently established stands than in older pine stands.

In conclusion, changes of seminatural forests by plantations of rapidly growing exotic species such as radiata pine, can lead to changes in soil microbial community, due to the inherent differences in litter quality. Mechanized forest operations in these plantations may exacerbate, even more, the soil microbial community change in favour of r strategist bacteria. The effect of these changes soil microbial biodiversity due to plantations forestry in the biogeochemical cycles needs more research.

(23)   Forest cover discontinuity shapes diversity of soil yeast communities

Oliver Röhl 1,2, Cláudia Carvalho 2, José Paulo Sampaio 2, Andrey Yurkov 3
1 Geobotany, Faculty for Biology and Biotechnology, Ruhr-Universität Bochum, Germany 2 Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal; 3 Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Brunswick, Germany

Forest properties have ultimate impact on aboveground diversity, especially on the so-called substrate-dependent taxa such as bryophytes and wood decaying fungi. Near-natural and unmanaged forests are often characterized by high heterogeneity of forest and soil cover as well as by high discontinuity. Forest alteration through management activities causes substantial changes also in belowground microbial communities and affects both prokaryotes and fungi, including yeasts.

In the present study, we assess the biodiversity of soil yeasts in Serra da Arrábida Natural Park, a Portuguese mountain limestone outcrop with two markedly different micro-climatic zones and distinct vegetation types, but largely composed by the same plant species: oaks (*Quercus faginea*, *Q. coccifera, Q. ilex, Q. suber*), Montpellier maple, juniper, wild olive, turpentine tree, tree heath, and strawberry tree. Differences in precipitation have a strong effect on vegetation cover, i.e. productivity and discontinuity. We sampled soils in three plots displaying a gradual change of (i) precipitation: humid forest, semi-humid chaparral forest, and dry maquis scrubland; and (ii) forest discontinuity: dense mature forest, chaparral forest, and patchy scrubland.

We characterized 613 yeast strains that were identified in 60 species, 17 ascomycetes and 43 basidiomycetes. These values are exceeding those reported for forests in Central Europe. A total of 8 (14%) yeast species occurred in all three sampling sites and 15 (25%) species were found in at least two sites. The majority of species (37 or 61%) were restricted to a single biotope.

Species richness estimations showed strong differences between plots and suggested a total of 80 yeast species to be expected from soils in Arrábida National Park. Scrublands are expected to have the highest diversity values, followed by humid and sub-humid forests. We hypothesize that wide occurrence of rare species increase species richness estimations for scrubland soil. Analyses of species-rank distribution and distribution of shared species showed that each plot yielded two most frequent (>10%) species, corresponding to 5-7% of the total richness. Number of species occurring with 1-10% frequency was declining from forest to chaparral and scrubland. Rare species (<1%) were extremely prominent (55% of the total richness) in soils under dry maquis scrubland. Differences in species occurrence result also in decreasing community evenness values from forest (J'=0.87) to scrubland (J'=0.8) vegetation.

Our results suggest that discontinuity of the vegetation cover may affect soil microorganisms, exemplified with yeast, shifting the community structure from the dominance of frequent species to the prevalence of minor and, possibly, transient species.

(24)   Root exudation depends on the type of mycorrhizal association

Rebecca Liese.1; Leuschner, C.1; Meier, I.C.1

1 Plant Ecology, Albrecht von Haller Institute for Plant Sciences, University of
Goettingen, Germany

Trees interact with their soil environment not only by the uptake of water and nutrients, but also by the release of root exudates, which increase the solubility of mineral nutrients, provide substrates for soil biota and stimulate the release of nutrients from soil organic matter. This feedback between trees and soil is mediated by symbiotic root associations, which presumably influence major root functions like root exudation and, thus, are controlling to some extent the plant-soil feedback. While a number of studies have investigated the influence of single, easy to culture root associates on root functions, the influence of diverse indigenous mycorrhizal communities on in situ exudation rates and rhizosphere processes in mature forest ecosystems remain understudied. Here we ask the question if tree species identity or mycorrhizal group association (arbusucular mycorrhization (AM) vs. ectomycorrhization (ECM)) has a stronger effect on carbon release through root exudation and subsequent rhizosphere processes. We selected each four AM and four ECM tree species from a mixed forest stand in Central Germany for our investigations. Root exudates were repeatedly collected during the growing seasons 2013 and 2014 with a novel technique for in situ exudate collection. In addition, rhizosphere soil (adhering soil method) was analysed for microbial biomass and net nitrogen mineralization rates. First results show an increase in root exudation rates in ECM tree species by three times in comparison to AM tree species. Possible causes for and consequences of this high increase in root exudation will be discussed.

(25)   Ammonia Oxidizing Bacteria community structure and richness under coniferous/deciduous tree species at three temperate forest sites

Sandrine Malchair and Carnol M.
Laboratory of Plant and Microbial Ecology
Department Biology, Ecology and Evolution
University of Liege, Belgium

Introduction:

Despite the crucial roles of soil microorganisms in ecosystem processes, numerous uncertainties subsist on the relationship between soil microbial diversity and function. Furthermore, the link between aboveground and belowground diversity remains also unclear. In European forests, at the beginning of the 19th century, plantations of Norway spruce and Scots pine for timber production were substantially increased. However, concerns were expressed with regard to the ecological risks posed by coniferous monocultures. The conversion of these monocultures into broadleaved or mixed stands has been suggested as a solution. Nevertheless, belowground effects of such a change in the dominant tree species is largely unknown, although bacteria regulate many soil processes and some groups, like ammonia oxidizing bacteria (AOB) are highly sensitive to environmental stresses.

Objectives:

The aims of this study were to investigate (i) AOB community structure and richness under several tree species, (ii) microbial/environmental factors influencing AOB diversity, (iii) the relationship between AOB diversity and the nitrification process.

Materials and methods:

Forest floor (Of, Oh) was sampled under Norway spruce, Douglas fir, European beech and sessile oak at three temperate forest sites. AOB diversity (community structure and richness) was assessed by PCR-DGGE and sequencing. Samples were analysed for microbial (net N mineralization, potential nitrification, basal respiration, microbial biomass, microbial or metabolic quotient) and environmental parameters (pH, total nitrogen, extractable ammonium, organic matter content and exchangeable cations).

Results:

AOB sequences retrieved in this study were related to different uncultured strains from soils, related to both *Nitrosospira*-like and *Nitrosomonas*-like sequences. AOB community structure and tree species effects on AOB diversity were site-specific. AOB community structure was influenced by environmental/microbial parameters, i.e. net N mineralization or organic matter content, regulating ammonium availability. AOB richness was not related to nitrification but a very weak correlation between potential nitrification and AOB community structure was observed.

Conclusions:

At larger spatial scales, site specific characteristics may be more important that tree species in determining AOB richness and community structure. However, within sites, tree species influence AOB diversity. The absence of a clear relationship between AOB diversity and nitrification points to a possibly role of AOB abundance, phenotypic plasticity or the implication of ammonia oxidizing archaea in this process.

(26)   In search for the link between growth of Norway spruce and the diversity of symbiotic ectomycorrhizal fungi

Sannakajsa Velmala[1], *Taina Pennanen*[1], *Tiina Rajala*[1]
[1]*Finnish Forest Research Institute, Jokiniemenkuja 1, Vantaa*
sannakajsa.velmala@metla.fi

Host related factors are known to influence the interaction between forest trees and the associated fungi. We have studied the interaction of Norway spruce (*Picea abies*) with the root associated ectomycorrhizal fungi. Our aim was to address how susceptibility to fungal infection and the composition and functionality of associated ectomycorrhizal fungal communities relates to spruce growth performance.

We found the host genotype to partly control the colonization of ectomycorrhizal fungal species (Velmala *et al.* 2013), but Norway spruce seedlings originating from differently growing seed orchards were colonized similarly by ectomycorrhizal fungi (Velmala *et al.* 2014 a, b). Single ectomycorrhizas had equal exoenzymes capacity regardless of the spruce origin, but the functional capacity varied highly according to the colonizing fungal species (Velmala *et al.* 2014 a). However, the short root architecture, which was found to be moderately heritable (Velmala *et al.* 2013), varied consistently between the fast- and slow-growing Norway spruce origins (Velmala *et al.* 2014 a, b). The seedlings of fast-growing origins had sparse and widespread rootlets that may lead to higher functional capacity compared to slow-growing seedlings.

It seems that Norway spruce does not show a strong genetic signal for within-population selection towards its mutualistic fungi at the species level. Formation of the associated ectomycorrhizal fungal community may be an effect rather than cause of seedling physiological state. The superior growth of fast-growing genotypes seems to be a consequence of resource allocation and optimal root structuring in the juvenile stage rather than the extent of colonization by ectomycorrhizal fungi. We accept that root physiological factors may subsequently lead to a higher capacity for symbiotic interactions in heterogeneous forest soil and a higher diversity and functionality of associated ectomycorrhizal fungi. An adequate and versatile means of nutrient acquisition is an important factor enabling fast growth, but might also provide the basis for positive feedback via enhanced relationships with mutualistic fungi.

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(27) What are the large-scale diversity, distribution and fate of Europe's forest mycorrhizas?

Sietsie van der Linde

Imperial college London, UK

We aim to fill a gap in our understanding of the wider diversity and distribution of mycorrhizal tree-fungi symbioses. We are expanding on previous sampling (for a PhD studentship and a Marie Curie fellowship) of ICP Forests, one of the most extensive biomonitoring plot networks on Earth in which the effects of pollution and changes in forest soil quality have been closely monitored. With support from the UK Natural Environment Research Council, we are now carrying out the first precise mapping of mycorrhizal fungi across Europe's major forest types: Beech, Scots pine and Norway spruce. At each intensively-monitored plot, we use optimised molecular ecology techniques to obtain DNA sequences from ectomycorrhizas. Up to now we have sampled over 50 plots in 14 European countries. Once we know who and where are the dominant fungi in Europe, we intend to combine the monitored data from the plots with the latest environmental change predictions to understand the processes that control these hidden symbioses and their likely fate in our changing world.

(28)   Anatomical-morphological identification of fine roots of the common European tree species

Tanja Mrak[*], Jožica Gričar, Peter Železnik, Hojka Kraigher

Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana

* corresponding author (tanja.mrak@gozdis.si)

Fine roots (≤ 2 mm) are the most dynamic part of the root system and represent the primary route for uptake of water and nutrients to the plants (Joslin et al. 2006). At the same time, turnover of the tree fine roots is one of the major carbon pathways in forests (Brunner et al. 2013). The ability to identify tree (fine) roots would enable analyses of root distribution of different tree species as well as linking of mycorrhizal symbionts to their hosts, thereby allowing for studies of belowground functional pathways. Root identification key of Cutler et al. from 1987, which is sold out, is mostly based on single specimen not regarding the possibility of certain anatomical character to change within the root (Trockenbrodt et al. 2001), e.g with decreasing diameter/root order. The key of Cutler et al. (1987) was also used for the preparation of the anatomical key of the most common European boreal and temperate ectomycorrhizal hosts in Agerer's Colour atlas of ectomycorrhizae (1987-2008). The comprehensive atlas of Kutschera & Lichtenegger (2002) is missing identification key and information on typical characteristics of the presented species are difficult to find. Up to our knowledge, there is no identification key available that would take into account also the morphological chracteristics of the roots. In the frame of EUFORINNO project (REGPOT no. 315982), we are investigating both anatomical and morphological identification approaches on roots of ten common European tree species to specify the most useful identification characteristics. These characteristics will be used to prepare an identification key for the studied species and if possible, extended to other woody plants. Roots of diameters less than 5 mm are analysed, taking into account also the most distal fine roots. Anatomy of roots is studied on transversal and longitudinal/radial sections, whereby especially root wood characteristics are considered and compared to characteristics of stem wood described in the existing stem wood identification keys. For morphology, characteristics such as diameter of lateral branches, branching pattern, colour, texture of the root bark or epidermis are evaluated as suggested by Pregitzer et al. (2002).

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(29)   Disentangling relationships between soil organisms and soil processes under different land-use intensity systems and climate change scenarios

Tiago Natal-da-Luz1, J. Raimundo1, L. Filho2, J. Bröring2, G. Ceola1,2, S.L. Stürmer3, D. Baretta2, O. Klauberg2, P.V. Morais1, J.P. Sousa1

1 IMAR-CMA, Department of Life Sciences, University of Coimbra, Portugal
2 Santa Catarina State University, Brazil
3 Regional University of Blumenau, SC, Brazil

The relation between soil biodiversity and ecosystem functioning is one of the Holy Grail's of Soil Ecology. The pathway "biodiversity–biological processes–ecosystem services" is well established for several organism groups, i.e., the importance of these groups in soil functioning is known. However, the level of biodiversity (structural, functional) needed for the system to function and to provide an ecosystem service (ES) at a desired rate (service providing units - SPUs) is still unknown. Furthermore, drivers like climate change and management have an influence on biodiversity and consequently on ecological processes and ES provision. Therefore is important not only to unveil the SPUs for several ES, but also to disentangle the effects of such drivers on biodiversity and ES provision. In this contribution a novel approach to tackle these aspects is presented, including manipulative experiments performed in two climatic zones (Mediterranean and Sub-tropical) and comparing two land-management systems (sustainable vs. conventional) at each zone. Mesocosm experiments are being conducted to improve knowledge regarding C and N cycles, emphasising key traits involved in resistance and resilience mechanisms under the influence of extreme climatic events (extreme rain and drought). Effects on soil fauna, soil microbial processes and community composition are being assessed. On a second phase, manipulations of soil fauna and arbuscular mycorrhizal fungi communities, aiming to create treatments with different functional dissimilarity levels, will be set up and similar parameters will be measured. It is expected to find higher structural and functional dissimilarities in the sustainably managed systems, enhancing traits of higher resistance and/or resilience against climate change. Additionally, it will be possible to understand the interactions between soil fauna and soil microorganisms in mediating soil processes related to C and N and to gain knowledge on which theoretical frameworks, mass ratio and niche overlap theories, could better explain these processes.

(30)   Determining environmental impacts on forest soil biodiversity's ability to sequester and store carbon

Uroš Radojević, MSc, Faculty of Applied Ecology Futura, University Singidunum, Belgrade

Jelena Milovanović, PhD, Faculty of Applied Ecology Futura, University Singidunum, Belgrade

Forests have a key role in climate change. A fair amount of anthropogenic greenhouse gas emissions results from changes within forests and changes from forest to other land uses. One of the most important forest carbon stocks is forest soil. Globally it is estimated that forest soil carbon stock may vary from equal to twice that of forest vegetation. Because of that it is important to have estimates of soil carbon and soil biodiversity which plays a key role in soil carbon sequestration. This can be done by either through repeated measurements or by application of dynamic models. Application and testing of soil carbon models typically require information about the amount and quality of litter input to the soil, the amount of carbon in different soils, environmental factors (like climate) and the changes in the amount of soil carbon over time. Although models need to be validated, their application provides quite a few benefits, especially since many countries have comprehensive and representative national forest inventories which can be used as input data for biomass turnover.

It is well known that environmental factors like temperature and humidity influence the forest soil microbial activity and in turn the forest soil carbon cycle. However, other factors, like forest management, can also have a significant impact on forest soil carbon cycle. To determine the extent of these types of impacts on microbial activity and associated forest soil carbon cycle an application of different types of models in combination with measurements and laboratory analysis can be used. In order to apply a model-based approach to soil carbon analysis it is first necessary to gather general information about the region in relevant aspects such as vegetation, climate, natural disturbances and management of ecosystems; analyze the available models for forest biomass and soil which will be used for the analysis (there are already available models like CO2Fix and Yasso which can be used); determine the availability of input information necessary for the models; decide upon the time period that the calculations will cover; determine the most suitable soil sampling methodology; set up a methodology to analyze microbial activity in soil samples. After this framework has been established following practical steps can be taken:

- choice of the forest biomass and soil carbon model to be used
- evaluation and possible improvement of the soil carbon model to be used in the application
- gathering necessary data (geographic, biomass, litter, soil, climate, land use, forest management) calculation units
- gather and analyze soil samples
- determination of initial soil carbon pools to be used in the calculations
- simulation of soil carbon cycling in the region of the application over the study period
- determining the impact of environmental factors on microbial activity and soil carbon
- result analysis

The final aim will be to determine the applicability of available soil and forest biomass models in determining the links between forest soil biodiversity and forest soil carbon cycle.

(31) Is influence of heavy forest machinery on fine roots observable in minirhizotron data?

Yasmine Pinuela Samaniego[1], Peter Železnik[2], Hojka Kraigher[2]

[1] Technical University of Madrid (UPM).

[2] Slovenian Forestry Institute

Impact of heavy forest machinery on soil properties is well described. Little is known about the influence of soil compaction by heavy machines on fine roots dynamics. The response of the belowground part of forest ecosystems on forest management is reflected on carbon and nutrient cycles: fine roots play important role in long-term changes in soil organic matter and ecosystem carbon balance. We observed impact of logging activities on fine root survival and longevity with minirhozotrons. The study was carried out in a Norway spruce (*Picea abies* (L.) H.Karst) stand situated on mountain region Pohorje in the area called Osankarica.

Minirhizotrons were installed on two subplots in and beside wheel rut and in undisturbed soil profile. There were no fine roots under the most adverse conditions (in the wheel ruts made by harvester and forwarder). In average, the highest fine root longevity was observed by wheel rut and the lowest in undisturbed soils.

The influence of heavy machinery on soils was clear and the responsible forest management should be essential to minimize damage of forest operations on soil part of forest ecosystem.

(32)   Root detection of various tree species using ground penetrating radar

Yasuhiro Hirano[1], Toko Tanikawa[2], Masako Dannoura[3], Keitarou Yamase[4], Kenji Aono[5] and Hidetoshi Ikeno[6]

[1]Graduate School of Environmental Studies, Nagoya University, Nagoya 464-8601, Japan
[2]Kansai Research Center, Forestry and Forest Products Research Institute, Kyoto 612-0855, Japan
[3]Graduate School of Global Environmental Studies, Kyoto University, Kyoto 606-8502, Japan
[4] Hyogo Prefectural Technology Center for Agriculture, Forestry and Fisheries, Shiso 671-2521, Japan
[5]The General Environmental Technos Co., Ltd. (KANSO TECHNOS), Osaka 541-0052, Japan
[6]School of Human Science and Environment, University of Hyogo, Hyogo 670-0092, Japan

Ground-penetrating radar (GPR) is a recent powerful tool to non-destructively detect coarse tree roots as hyperbolas in a radar profile. In mixed forests, questions will arise whether GPR can differentiate roots of various tree species using GPR signals. We have scanned roots of *Cryptomeria japonica*, *Pinus desiflora*, *Quercus serrata* and rhizome of *Phyllostachys pubescens* which buried at 30 cm depth in sandy experimental conditions using 900 MHz GPR. A steel pipe buried at the same condition was used as control.

As a result, the hyperbolas reflected buried roots were clearly seen all plant species which were similar to a steel pipe. The curvatures of hyperbolas were not different among plat species, suggesting that GPR signals cannot distinguish among plant species. On the other hand, GPR indexes such as sum of amplitude area (A) or of time intervals (T) for all reflection waves were positively correlated with root diameter across plant species. These results suggest that root diameter can be estimated across plant species, but the scanning condition of soils and roots such as water content and orientation should be considered.

(33)   How do roots influence soil properties and biotic processes across French agroforestry landscapes?

Yogan Monnier, Alexia Stokes
INRA, UMR AMAP, 37/PS1, Bd de la Lironde, 34 398 Montpellier cedex 5

In French agricultural landscapes, conventional farming can amplify soil erosion. Recent policies have encouraged agroforestry practices that are believed to provide a number of ecosystem services and improve biodiversity. However fundamental and applied research is still required to determine how best to plant and manage, depending on land use, climate and topography. Within this context, our project investigates the ecosystem services provided by crop/forest systems, in relation to soil structural stability. We chose to study the following variables: stability of soil aggregates, root traits and distribution, microbial activity and microbial metabolic diversity. The project involves eight field sites representative of different agro-forests which have distinct ages and pedoclimatic characteristics. On each site, a systematic sampling was performed to assess the influence of the tree row, the distance to the trees and the perennial plant cover on the variables listed above. The relationship between soil aggregates and functional traits of plants and soil microbes is studied with substantial field measurements involving a large network in French agroforests. The preliminary results of this project suggest that the tree row and the proximity of trees are beneficial for soil aggregate stability. This result may be related to the quantity of roots. The magnitude of these effects seems however to depend on the characteristics of the agroforestry system. The results of this research should allow stakeholders and agroforesters to better determine adapted spatial management strategies with regard to plant root traits and erosion risks.

(34) Earthworm stage structured population dynamic model: an ecological category approach

Željka Lončarić,[a] , Hackenberger, D.K.[a], Hackenberger, B.K.[a*]

[a] J. J. Strossmayer University, Department of Biology, Cara Hadrijana 8/A, HR-31000 Osijek, Croatia
Željka Lončarić zloncaric@biologija.unios.hr,
Davorka K. Hackenberger davorka@biologija.unios.hr,
Branimir K. Hackenberger hack@biologija.unios.hr

Earthworms are known as one of the key organisms in soil ecosystems due to biomass and functions they provide. Their population dynamics is influenced by many abiotic and biotic factors. However, living in the soil hinders the observation of their populations. One of the promising approaches to earthworm population dynamics research is modeling. The aim of this study was to construct a simulation model for the prediction of the abundance of earthworm population as a function of soil temperature and soil water content. The basis for the model was field data from two sampling occasions. Sampled earthworm species were grouped into ecological categories (epigeic, endogeic and anecic) according to their ecological and morphological traits. Furthermore, Lefkovitch discrete model of stage structured population dynamics was adapted to an earthworm life cycle parameters for all three earthworm ecological category and calculated with a weekly time step. Simulation of fluctuating abiotic factors at various soil depths was obtained by using the soil temperature and moisture model. Simulated values were compared with field measured data and mostly corresponded with the number of sampled earthworms with a few under- and overestimations. In the present model, as an additional parameter of population regulation a possibility of additional mortality was added. Overall average additional mortality was higher for juvenile (16.25%) than for the adult (11%) earthworms. Across the functional groups the highest additional mortality was set for epigeic earthworms (19%), while endogeic and anecic earthworms had the same additional mortality (11.14% and 11.25%, respectively). Presented approach of modeling the population dynamics of earthworms at the level of ecological categories seems promising and could reduce the laborious field work and determination of earthworm to a species level. This model could be suitable for the application in various aspects of environmental management. The inclusion of population modeling in ecological monitoring of earthworms would indicate changes in population structure and changes in the amount of various pressures on earthworm populations with less sampling.

(35) How to better describe spatial heterogeneity of fine root distribution in tree ecosystems?

Zhun Mao[1*], Rémi Cardinael[2], Christian Dupraz[2], Thomas Cordonnier[1], Alexia Stokes[3], Christophe Jourdan[4]

1 IRSTEA, UR EMGR, Centre de Grenoble, 2 Rue de la Papeterie, BP 76, 38402 Saint-Martin-d'Hères, France ;
2 INRA, UMR System, Bâtiment 27, 2 Place Viala, 34060 Montpellier, France ;

3 INRA, UMR AMAP, Boulevard de la Lironde, 34398 Montpellier Cedex 5, France ;
4 CIRAD, UMR Eco&Sols, Bâtiment 12, 2 Place Viala, 34060 Montpellier, France
*For correspondence: Dr. Z. Mao; tel.: +33 4 76 76 28 84; fax: +33 4 76 51 38 03; email address: maozhun04@126.com.

In tree ecosystems, tree fine roots (defined as those of diameter ≤ 2 mm) comprise only a small proportion of total biomass, but play the most active and fundamental role in both tree functioning (via water and nutrition uptakes) and carbon flux dynamics (via respiration, exudation and high turn-over rate). These processes have a beneficial effect on the promotion of underground biodiversity, as fine roots with their rhizospheres can provide "cradle effect" for sheltering and nourishing soil micro- and macro-organisms in both direct (e.g. mycorrhizal fungi growth on roots) and indirect (e.g. creating soil organic matter) manners. As a result, in order to better explore underground biodiversity repartition and evaluate its service, a reliable characterisation of the spatial distribution of fine roots is indispensable. The spatial distribution of fine root can be served either as a proxy of repartition of certain fauna or as an environmental factor to be correlated with biodiversity indicators. However, our knowledge concerning fine roots distribution is still limited, partially due to the lack of effective root modelling and data analysis methods.

This proposal aims to introduce and highlight a set of methodologies suitable to quantitatively describe root spatial distribution. The methods are statistically based and easy to use in conjunction with conventional root sampling techniques (e.g. root trench, coring). Fine root density from trees at a given point can be modelled by a modified logistic function parametrised by tree dimension and position, presence of obstacles between a tree and the given point, and root competition level with roots from understory species. For fine root vertical distribution, by introducing a recently published piecewise linear model', namely "hockey stick model," an ecological threshold served to distinguish shallow rooting zone and deep rooting zone, might be thus detected with statistical diagnostic. Applying a log-normal function and Gompertz function allows us to better fit the shape of root diameter spectrum (cumulative root density with increasing diameter). Regarding the phenomenon that fine root distribution exhibits a pattern of patchiness (or namely root nuggets), application of geo-statistical approaches allows us to better quantify aggregation and dispersion rate of roots within/amongst nuggets.

In the ongoing projects ANR ECOSFIX (Ecosystem Services of Roots – Hydraulic Redistribution, Carbon Sequestration and Soil Fixation) and ARANGE FP7 (Advanced multifunctional management of European mountain forests), these above methods have been successfully applied to several French tree ecosystems, varying from heterogeneous mixed mountain forests (with low intervention intensity), to homogenous agroforests and plantations (with high intervention intensity). In the framework of Cost Action Biolink, it might be therefore particularly interesting to have these novel root analysis methods applied toward new root data, in order to better evaluate the state and roles of belowground soil biodiversity.