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RELATIVE IMPORTANCE OF FOREST STRUCTURE AND COMPOSITION FOR SOIL  
TROPIC GROUPS DIVERSITY ALONG ALTITUDINAL GRADIENTS IN THE FRENCH ALPS

Laureline LECLERC



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## Introduction

Forest ecosystems provide numerous functions such as primary production, protection against soil erosion and regulate water and element cycles, notably by allowing carbon storage. Although the area occupied by forests in Europe has increased over the last century (FAO, 2020) due to changes in land use and in particular through agricultural abandonment (Feuillet et al., 2020), forests are threatened by climate change, particularly in mountainous regions where climate change is accentuated (Ranasinghe et al., 2021; Rottler et al., 2019). Changes in temperature, precipitation, atmospheric CO<sub>2</sub>, pest damage and fire threaten the structure and functioning of forests (Moreno et al., 2018; Morin et al., 2018; Orlova-Bienkowskaja et al., 2020; Senf et al., 2018 in Bednar-Friedl et al., 2022). Moreover, mountains offer the opportunity to study forests along elevation gradients to explore the potential effects of climate change with a space for time approach.

Trees are key elements of forest ecosystems, but the functioning of these ecosystems is also driven by a myriad of other organisms and multiple trophic levels (Semeraro et al., 2022; Six et al., 2006; Smith et al., 2015). Because of the strong ecological linkages between the trees and the soil biodiversity (Wardle et al., 2004), multitrophic soil biota are key elements for the forest ecosystems functioning (Schuldt et al., 2018). These organisms spend all or part of their life cycle in the soil and are involved in both soil chemistry and physical structure (Pavao-Zuckerman, 2008). Indeed, micro-organisms, meso- and macro-fauna of the soil are included into the functioning of biogeochemical cycles through the decomposition of organic matter (OM). A soil trophic network, made up of different trophic groups, breaks down organic matter at different scales and thus enables the recycling of nutrients and is the driving force behind primary production. In addition to the degradation of OM, so-called engineer organisms, such as earthworms, modify the soil structure by creating galleries that allow both the circulation of water and air necessary to maintain aerobic environments. High species richness within functional groups supports the capacity of an ecosystem to resist environmental stresses and perturbations, since it multiplies the chances that within the same functional group, by functional redundancy, certain species survive and maintain the functioning of the ecosystem (Biggs et al., 2020).

Climate change is a threat not only for trees but also for soil biodiversity (Henneron et al., 2015; Santonja et al., 2017). Indeed, temperature and water availability are important factors for these organisms (Aupic-Samain et al., 2021; Eggleton et al., 2009 & Kardol et al., 2011 in

Indoria et al., 2020). An increase in temperature, a change in precipitation and especially repeated droughts or frost episodes could affect the physiological functioning of soil biota and modify biodiversity. The soil biodiversity is also modulated by the soil characteristics. For instance, the availability of nitrogen in the soil is a limiting factor, the pH of a soil, and its water retention capacity also select organisms that are able to survive in a site (Calderón-Sanou et al., 2022). However, in forest ecosystems, the forest structure and composition are additional key variables controlling soil biota diversity (Penone et al., 2019; Tinya et al., 2021; van der Linde et al., 2018). Given the high biomass stored in trees and their huge input to the soil, the forest structure can have important effects and thus could modulate the effect of abiotic factors.

In forests, trees provide both nutritional resources and habitats for soil organisms. On the one hand, living trees are subject to herbivory and their roots can be consumed by phytophagous insects for example. On the other hand, most trees shed large quantities of leaves, which form the bulk of the litter and organic matter that is decomposed by soil biota. Litter chemical composition determines the presence, in the soil, of organisms able to decompose it. The activity of organisms is limited by the availability of nitrogen in the soil, so litter with a low C/N (carbon to nitrogen) ratio is more easily degraded by organisms than litter with a high C/N ratio (Cleveland & Liptzin, 2007; Wall, 2005). Thus, plants composition, and especially that of tree layer, which constitutes an important part of forests biomass, determines soil biodiversity (Harrison & Cornell, 2008; Tedersoo et al., 2016). In addition, trees and some micro-organisms live in mutualistic or parasitic associations. For instance, some fungi form mycorrhizae and many bacteria live in the rhizosphere or phyllosphere of trees, so that the diversity of the tree layer provides a variety of symbiotic conditions for these micro-organisms (van der Linde et al., 2018).

Dead trees are also an essential compartment for forests functioning. Indeed, deadwood has stored a large quantity of nutrients which will be gradually returned to the soil, thus preserving its fertility (Palviainen et al., 2010). Saprophytic organisms live in deadwood and consume it. 20% to 25% of forest-dwelling species are saprophytic and depend on deadwood (Schuck et al., 2004). Deadwood diversity, *i.e.* the species of the dead tree, its moisture content, the stage of decomposition or whether it is a snag, a stump or a log, corresponds to many combinations with which particular saprophytic species are associated (Lassauce et al., 2011).

In general, links between forest structure and soil organisms from multiple trophic levels have been relatively little studied and we do not know the relative importance of abiotic factors *vs.* forest structure on soil biodiversity. The effect of forest cover on soil biodiversity is sometimes negative (Bouget et al., 2014; Tedersoo et al., 2016), positive or non-existent

depending on studies and taxonomic groups (Henneron et al., 2015; Janssen et al., 2018). In contrast, the importance of deadwood for saproxylic biodiversity is widely documented (Bouget et al., 2014; Brin et al., 2011; Lassauce et al., 2011; Moll et al., 2021; Parisi et al., 2018). An increase in tree volume or deadwood is expected to increase soil biodiversity that depend on living or dead trees as a nutrient or habitat resource, *e.g.* mutualistic or parasitic micro-organisms of trees (Spake et al., 2016) and saproxylic organisms (Lassauce et al., 2011). Furthermore, as soil C/N appears to be related to tree leaf content in carbon and nitrogen (Dawud et al., 2017), the tree leaf C/N can especially affect soil biodiversity. The area-species theory, which has been extended by a hypothesis on the link between the amount of energy and biodiversity (Wright, 1983), argues that when the amount of habitat or resource increases, so does the biodiversity. Furthermore, biodiversity also tends to increase with the diversity of habitats and resources encountered in an ecosystem according to the habitat and resource heterogeneity hypothesis (Stein et al., 2014; Tews et al., 2004). In the soil, as habitat and resources overlap, it can be considered that as the diversity of living trees and deadwood increases, so does the associated biodiversity.

Improving our understanding of the relative importance of abiotic factors and forest structure in controlling soil biodiversity is thus key given the limited number of studies exploring this question. This could provide natural area managers with means of action to conserve biodiversity or enable to develop predictive models of climate change impacts. Here we propose to explore this question building on a large-scale observatory network across the French Alps (Orchamp). The study of soil organisms has long been a tedious exercise due to the difficulty of accessing soil compartment, observing the numerous and sometimes very small organisms and the lack of taxonomic knowledge about it (Decaëns, 2010). Therefore, studies on soil biodiversity have often been carried out on certain species or large functional groups or on restricted spatial scales. Recent environmental DNA techniques address these difficulties by facilitating taxon recognition for much larger sampling efforts (Zinger et al., 2016). Calderón-Sanou et al. (2022) used a metabarcoding approach on soil sample from Orchamp plots to have an evaluation of biodiversity for multi-taxon and trophic groups. They showed that across large elevation gradient covering multiple ecosystem type from forests, grasslands, up to nival ecosystems, abiotic variables associated with the quantity of energy and resources as well as the variables studied related to the physiology of organisms are the most important predictors of soil biodiversity. Here we propose to build on Calderón-Sanou et al. (2022) and to focus on the forest plots of the Orchamp network over 16 elevation gradients in order to assess the relative importance of climate and soil *vs.* forest features (in term of both structure and forest

diversity) for the diversity of 37 soil trophic groups. More specifically we test the following hypotheses :

- (i) Climate and soil are more important than forest structure and composition for the diversity of soil trophic groups.
- (ii) Forest variables should be more important for trophic groups that feed directly on organic matter from living or dead trees (*i.e.* decomposers, detritus feeders and plant symbionts) than for groups at higher trophic levels.
- (iii) The tree layer influences the diversity of soil trophic groups. (iiia) A higher heterogeneity of forest structure and composition should favour soil biodiversity. (iiib) The diversity of tree symbionts or soil groups directly consuming litter or wood increases with the amount of living, dead and easily decomposable tree leaves.

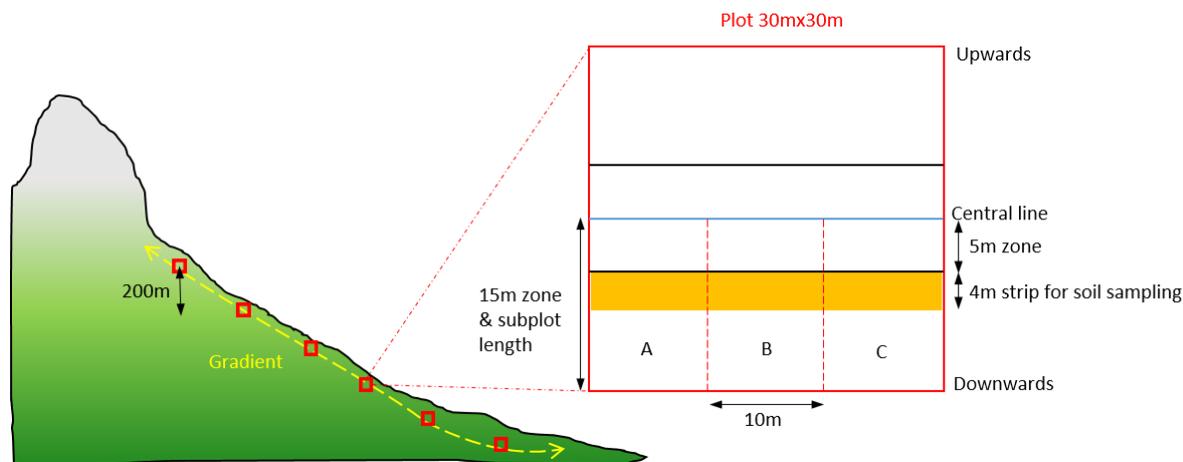
## **Material and Methods**

### **Study sites**

Data were collected between 2016 and 2022 along 16 altitudinal gradients belonging to the long-term observatory Orchamp (<https://orchamp.osug.fr/>). These gradients are distributed throughout the French Alps (ca. 40,500 km<sup>2</sup>) and encompass a variety of climatic, soil and vegetation conditions. Each altitudinal gradient is composed of 4 to 9 plots of 30x30m, with a homogeneous slope and exposure and separated by about 200m (Fig. 1). Only data from the 48 forest plots were considered in this study. The BD Forêt® V2 cartography of the French National Institute for Geographic and Forestry Information (IGN) was used to select plots located in forest. Plots belonging to areas described by the BD Forêt as 'closed forest' or 'open forest' (cover rate greater than or equal to 25%) were retained.

### **Experimental design and field measurements**

Figure 1 describes the organisation of each plot. A plot is divided into three sub-plots (A, B and C, Fig. 1) and a central line delimits the upper and lower parts of the plot.



**Figure 1** : Experimental design of the Orchamp observatory project. A gradient is composed of 4 to 9 plots ranging from low to very high altitude (280–3160m). Subplots used in this study correspond to the 150m<sup>2</sup> zones named A, B and C.

### *Forest features metrics*

The dendrometric protocol (live trees and deadwood) is an adaptation of the protocol for dendrometric monitoring of forest reserves (Protocole de Suivi Dendrométrique des Réserves Forestières (PSDRF) (RÉSERVES NATURELLES DE FRANCE et al., 2012)).

### *Livewood*

The diameter of live trees is measured at 1.30m (Diameter at Breast Height - DBH) and their species is identified with the following conditions. Live trees with a diameter greater than 30cm are surveyed in the entire plot. Live trees with a diameter between 7.5cm and 30cm are only surveyed in the area 5m upwards and downwards of the central line. Up to 1.50 m upwards and downwards of the central line, all live trees (taller than 1.3m in height) are surveyed regardless of their diameter.

### *Deadwood*

Three types of deadwood are recorded: fallen deadwoods (logs), standing deadwoods (snags, height  $\geq 1.30\text{m}$ ) and stumps (height  $\leq 1.30\text{m}$ ). For each piece of deadwood, the species is identified where possible, diameter measurements are taken to calculate the volume and each piece of deadwood is classified according to the degree of decay of the wood (hard, less than 50% rotten, more than 50% rotten). All deadwood with a diameter of 30cm or more is counted in the whole plot (Fig. 1). In the five-metre zone from the central line upwards and downwards

(Fig. 1), stumps and snags with a diameter comprised between 7.5cm and 30cm are also measured (average diameter for stumps and diameter at 1.30m for snags). Finally, along the central line (Fig. 1), deadwood pieces on the ground, intersecting the line and with a diameter comprised between 7.5 cm and 30 cm, are measured.

### *Soil sampling*

Soil sampling was carried out by Orchamp consortium under the supervision of W. Thuiller (Leca CNRS). Calderón-Sanou et al. (2022) describe the protocol in detail. Soil samples were collected at the end of the summer season from a 4m-wide strip in the lower part of the plot (Fig. 1). In each sub-plot (2×2 m), approximately 10 soil cores of 5cm-diameter were taken from 1 to 16 cm in depth and homogenized to obtain 1 sample per subplot *i.e.* 3 samples per plot (Fig. 1).

### **Environmental variables and soil biota**

To test our hypothesis, we defined four groups of explicative variables related to local (i) climatic conditions, (ii) soil conditions, (iii) forest quantity features and (iv) forest diversity features. Three variables were selected for each group of variables so that all 12 variables were not too correlated with each other (correlation coefficient < 0.7 (Zuur et al., 2010)) (see Annex 1 for information about environmental variables and Annex 2 for correlation coefficients between environmental variables).

### **Forest structure and composition metrics**

Pairs of variables were constructed to describe the forest by subplot. One variable belongs to the forest quantity features group of variables (basal area, deadwood volume and trees leaf CWM) while the other variable belongs to the forest diversity features group of variables (Gini coefficient of basal area, deadwood diversity and tree species diversity).

The tree volume and canopy cover are characterised by basal area ( $m^2/ha$ ), which is the sum of the cross-sectional areas of the trunks at 1.30m per hectare. The Gini coefficient calculated from the trunk DBH values gives the heterogeneity of tree sizes. The Gini coefficient varies between 0 (all trees diameters are equivalent) and 1 (maximum differentiation of tree diameters). For instance, Pach and Podlaski (2015) studied forests in Central Europe and find that the Gini coefficient varies from 0.31 to 0.48 in managed forests where trees tend to be of similar age

and diameter. In contrast, in unmanaged forests where tree sizes are more heterogeneous with notably big-sized trees, the Gini coefficient varies from 0.33 to 0.73.

Deadwood was characterised by the total volume and diversity of deadwood pieces. The total volume of deadwood corresponds to the sum of the volumes of logs (diameter > 30cm), stumps and snags that were obtained from the diameters and lengths or heights of each piece of deadwood and the value obtained from the deadwood on the ground surveyed on the central line (diameters between 7.5cm and 30 cm) according to equation [8] of Marshall, Davis and LeMay (2000) which allows to extrapolate a volume per hectare from a transect sampling. Deadwood diversity is characterised by a richness of combinations of deadwood characteristics *i.e.* the number of combinations accounting for tree species, deadwood types (log, snag or stump) and the deadwood degree of decay.

The community weighted mean of trees leaf C/N ratio is obtained by summing the trait value of each species weighted by each species abundance (measured by their respective basal area). The community weighted mean of trees leaf C/N is an indicator of the average quality of the litter produced mainly by abundant tree species. The higher the trees leaf C/N, the less easily the leaves can be decomposed. Furthermore, the diversity of tree species is calculated with the exponential of the Shannon index (Hill number with  $q=1$ ) and gives the “effective number” of tree species (*i.e.* the number of equiprobable species giving the same diversity value as the observed distribution, Chao et al. 2014).

## **Climate**

The climatic data comes from the SAFRAN-Crocus models (Durand et al., 1993; Vernay et al. 2022) and are retained over a period of 10 years prior to the sampling of plots, *i.e.* between 2008 and 2018 approximately. The three climatic variables retained are mean annual temperature, mean annual Freezing Degree Days (FDD) and mean annual Climatic Water Stress (CWS). The sum per year of the daily average temperature, in the 1<sup>st</sup> cm of soil, when it is below 0 gives the annual FDD. The FDD values are negative, the more negative the FDD, the greater the physiological stress due to frost. The CWS is an adaptation of the climatic water deficit (CWD; Stephenson, [1998](#)) to approximate the intensity of water stress for organisms. The CWS is calculated as the difference between the water supply from precipitation and snow and the potential evapotranspiration (PET) calculated according to the Penman-Monteith equation (Vannier & Braud, [2012](#)). When the water supply counterbalances the PET, the

potential water stress is considered negligible and the CWS equals zero. The lower the CWS values, the higher the potential water stress is.

## **Soil**

Data from the soil samples were taken from work carried out by Calderon-Sanou et al. (2022) and Martinez-Almoyna et al. (2020). 15g of each soil sample was used to extract environmental DNA (eDNA). Six DNA markers were used to detect numerous taxonomic groups: eukaryotes (mites, nematodes, Enchytraeidae, earthworms and protists) and bacteria using universal markers, and fungi, insects, springtails and oligochaetes respectively using specific markers. The environmental DNA analysis methods used allowed the taxonomic composition in terms of Molecular Operational Taxonomic Unit (MOTU) of each sample to be defined. Details of the DNA markers and the procedure used are available in Calderón-Sanou et al. (2022).

The soil pH, soil organic matter and soil C/N ratio were described from the rest of each soil sample, sieved to 2mm according to the method given by Martinez-Almoyna et al. (2020).

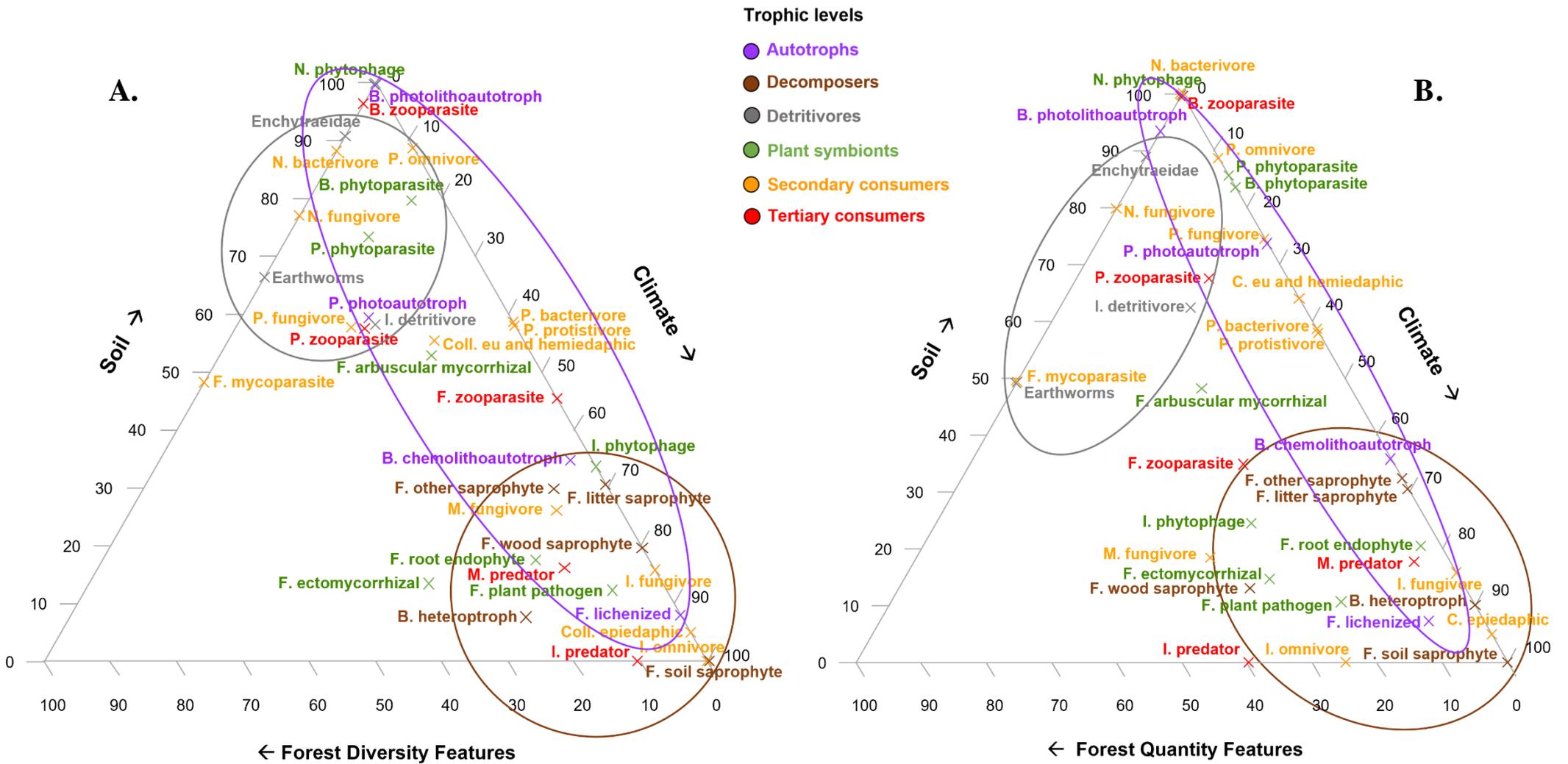
## **Diversity of trophic groups**

The work of Calderón-Sanou et al. (2022) also allowed trophic groups to be associated with MOTUs detected by the six eDNA markers. The different databases and references used for these assignments are detailed in Calderon-Sanou et al (2022). Using the R package "metabaR" (Zinger et al., 2021), 36 trophic groups were identified in the 48 forest plots considered. These trophic groups characterise separate trophic levels into the major taxonomic groups of fungi, protists, bacteria, insects, mites, springtails, nematodes, Enchytraeidae and earthworms (Calderón-Sanou et al., 2021 & 2022). The effective number of MOTUs was calculated per subplot as the exponential of the Shannon diversity index (Hill number with  $q=1$ ). This diversity calculation allows under representation of MOTUs with low read counts, which may correspond to artefacts related to the eDNA extraction method. Calderón-Sanou et al. (2020) showed that the use of the Shannon diversity index for eDNA data gives reliable estimates of biodiversity.

## Statistical analysis

All models performed are linear mixed effects models with a nested random effect *i.e.* intercept varying among gradient and plot within gradient. The diversity of each soil trophic group does not respond in the same way to environmental variables. Therefore, first analyses allowed selecting the best model for each trophic group and each of the 4 groups of environmental variables according to 3 types of transformations of the environmental variables (linear, logarithmic or polynomial of order 2). The "dredge" function of the "MuMin" package (Bartoń, 2022) was used to select from 0 to 3 terms per model and per group of variables by imposing the choice of a single transformation per variable. Then to estimate the relative importance of the four groups of variables, a full model is fitted per trophic group with the variable previously selected for each group of variables. Normality and homoscedasticity of residuals were checked graphically for each model. Pseudo-R-squared for mixed-effect models ("MuMin" package) were also calculated: the marginal r-squared (R2m) takes into account only fixed effects while the conditional r-squared (R2c) takes into account both fixed and random effects. From the full model, the importance of each variable or group of variables is calculated using the "explain" function of the "DALEX" package and the "feature\_importance" function of the "ingredients" package (both packages from Biecek, 2018). These functions calculate features importance from the change in the Root Mean Square Error (RMSE) value when permuting the data. The number of permutations was set to 499. The importance value of a variable (or group of variables) is calculated as the average of the 499 differences between the RMSE of full models without the variable (or group of variables) in question and the RMSE of the complete full models.

## Results



**Figure 2 :** Importance of **A.** Forest diversity features and **B.** Forest quantity features compared to Soil and Climate variables in explaining soil trophic groups diversity. Values are percentage of the contribution of the 4 groups of variables to the explanatory power of trophic groups diversity. Decomposers are more influenced by climate than by soil characteristics (brown ellipses). Conversely, soil is more important for detritivores than

climate (grey ellipses). Autotrophs are only slightly influenced by forest variables (purple ellipses). Letters indicate broad taxonomic groups : Bacteria (B.), Fungi (F.), Protozoa (P.) and Metazoa i.e. Collembola (C.), Insects (I.), Mites (M.), Nematodes (N.).

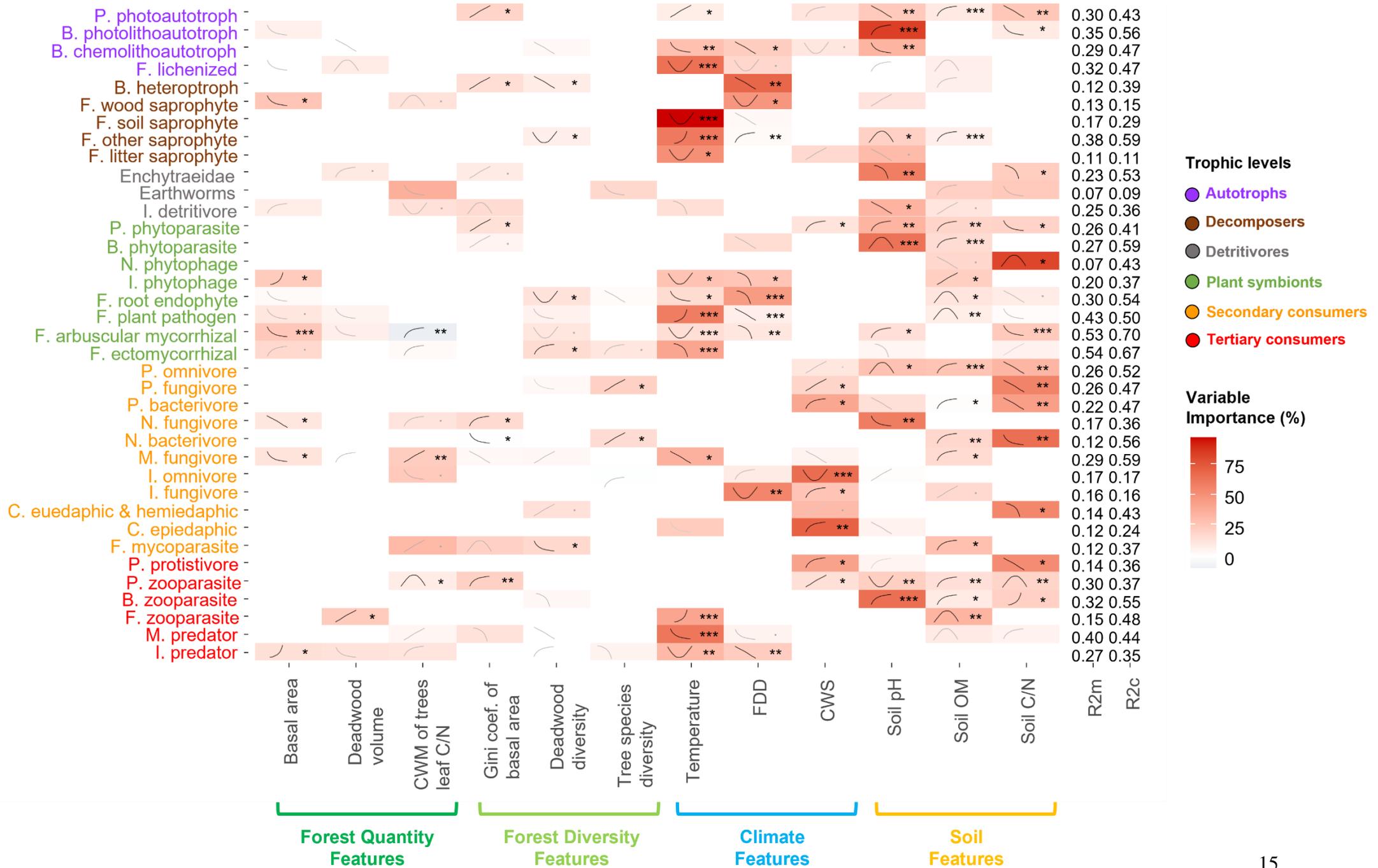
## Relative importance of forest, climate and soil features

For most of the trophic groups studied, the variables associated with the forest are, in agreement with our first (i) hypothesis, less important for their diversity than the climatic and edaphic variables considered (Fig. 2, Table 1). On average, climate explains 39% while soil accounts for 42%, forest quantity variables and forest diversity variables explain respectively 10% and 9% of the diversity of a trophic group. The importance of forest variables sometimes exceeds 20% of the explanatory power of models which explain the diversity of certain trophic groups rather well. More than 30% of the diversity of predatory insects ( $r^2_m=0.27$ ,  $r^2_c=0.35$ ), ectomycorrhizal fungi ( $r^2_m=0.54$ ,  $r^2_c=0.70$ ) and fungivorous mites ( $r^2_m=0.29$ ,  $r^2_c=0.59$ ) is explained by forest features which also account for about 20% of the diversity of plant pathogenic fungi ( $r^2_m=0.43$ ,  $r^2_c=0.50$ ), arbuscular mycorrhizal fungi ( $r^2_m=0.53$ ,  $r^2_c=0.70$ ) and zooparasitic protists ( $r^2_m=0.30$ ,  $r^2_c=0.37$ ) (Fig. 2).

Contrary to what we assumed in our hypothesis (ii), decomposers, detritivores and plant symbionts do not appear to be more influenced by forest variables than other trophic levels (Fig. 2, Table 1). However, within these trophic levels, trophic groups do not respond in the same way. Vegetation-dependent groups such as pathogen or mycorrhizal fungi (ectomycorrhizal and arbuscular) appear to be more influenced by forest variables (Fig. 2, Table 1). Furthermore, decomposers are more influenced by climate than by soil characteristics (Fig. 2). Conversely, soil is more important for detritivores than climate (Fig. 2). Autotrophs, which are not specifically dependent on trees, are almost not influenced by forest variables (Fig. 2).

Furthermore, a dichotomy between taxonomic groups can be observed. Mites, as well as most fungi and insects, are more strongly related to climatic variables than to soil variables. Conversely, soil variables are more important than climatic variables for protists, nematodes, oligochaetes (earthworms and Enchytraeidae).

**Table 1 :** Effects of forest, climate and soil features on soil trophic groups diversities. Curves represent the type of relation between trophic groups and environmental variables ( $\swarrow$  for positive or  $\searrow$  for negative linear,  $\smile$  for positive or  $\frown$  for negative logarithmic and  $\cup$  or  $\cap$  for polynomial relations). Greys and black symbols means respectively non-significant and significant variables effects with associated p-values thresholds: ‘\*\*\*’  $\leq 0.001$  ; ‘\*\*’  $\leq 0.01$  ; ‘\*’  $\leq 0.05$  ; ‘.’  $\leq 0.1$  ; ‘ ’  $\geq 0.1$ . The importance of each variable is given in shades of red by its contribution to the explanatory power of the full model (in percentage of RMSE loss).



## Responses of the trophic groups to forest features

The variance explained by the models constructed varied from 9% for Earthworms to 70% for arbuscular mycorrhizal fungi when accounting for fixed and random effects (R2c; Table 1).

Forest variables had no effect on the biodiversity of only eight out of 37 trophic groups: protistivore, omnivore and bacterivore protists, fungi saprophyte of soil and litter, epiedaphic springtails, phytophage nematodes and fungivore insects (Table 1). Forest diversity variables are significantly related to the diversity of thirteen out of 37 trophic groups while the variables of forest quantity are significantly related to the diversity of ten out of 37 soil trophic groups (Table 1). The magnitude of these significant effects is overall low (Table 1). Magnitudes arise from full models and are rounded to the tenth of 'effective' MOTUs.

When the Gini coefficient increases by 0.1 unit, the diversity of heterotrophic bacteria, photoautotrophic plant and animal parasites protists, fungivore nematodes increase by 0.1, 0.2, 0.2, 0.3 and 0.1 respectively but bacterivore nematodes decrease by 0.1. When the diversity of deadwood increases by 1 additional combination, the diversity of heterotrophic bacteria and mycoparasitic fungi decreases by 1 and 0.4 respectively while the diversity of ectomycorrhizal fungi increases by 0.4. Other saprotrophic and endophytic root fungi show a polynomial relationship with the diversity of deadwood with a minimum biodiversity. The diversity of fungivore protists and bacterivore nematodes increases by 1.2 and 0.4 respectively when the effective number of tree species increases by 1.

When basal area increases by 1m<sup>2</sup>/ha, the diversity of wood saprophytic fungi, arbuscular mycorrhizal fungi, fungivore mites, fungivore nematodes decreases by 1.1, 0.5, 1.1 and 1 respectively. The diversity of phytophage and predator insects increases according to a polynomial relationship with basal area. The diversity of animal-parasitic fungi increases by 1 with 1m<sup>3</sup> additional volume of deadwood. Arbuscular mycorrhizal fungi and fungivore mites increase by 0.5 and 1 respectively when the C/N tree increases by 1.

Our (iii) hypothesis is partially verified. Forest features have a significant effect on only some soil trophic groups (Table 1). The diversity of most groups increases with the diversity of forest composition and structure (Gini coefficient, deadwood and tree diversity) (Table 1). In contrast, the diversity of plant symbionts, litter or deadwood consumers is not significantly enhanced by increasing basal area, deadwood or low C/N tree litter (Table 1).

## Discussion

### Climate, soil vs forest features

We found that the forest variables studied were less important than climatic and edaphic variables, which has already been shown in other studies (Penone et al., 2019; Tinya et al., 2021). Indeed, thermal and hydric conditions exert an important ecological filter as they directly affect the metabolic functioning of organisms (Berg & McClaugherty, 2014). The soil compartment is the living environment of soil organisms, that are distributed spatially for instance according to their affinity for more or less acid or basic substrates (Siles & Margesin, 2016). The quantity of organic matter and the isometry of the nutrients it contains are also important as a nutrient resource for soil organisms (Vos et al., 2013). Soil organic matter comes from tree vegetation, but also from herbaceous plants and animal feces and necromass. These different origins of soil organic matter as well as the nature of the soil (granulometry and parent rock (Roy et al., 2013)) could for example explain why the C/N of soil organic matter was more important than the C/N of the leaves of the most abundant trees for soil biodiversity in our study.

The experimental set-up used for this study, consisting of altitudinal gradients (from 280m to 2100m) located in several bioclimatic sectors from the south to the north of the French Alps, is particularly well suited to highlight the importance of climatic conditions for organisms. However, we showed that the importance of forest variables for soil biodiversity is not negligible especially for certain soil trophic groups despite the quite low diversity of forest conditions. Indeed, the range of variation of forest conditions is quite limited, maybe because of the type of forest management. Most of the forests studied are managed as uneven aged stands, which results in a different structure and biodiversity than even-aged stands or strict forest reserves (Paillet et al., 2010). Deadwood is a good example of the low variation observed on the plots studied. For example, the average volume of deadwood on the studied plots is 5.67 m<sup>3</sup>/ha (Appendix 0) while the total deadwood volume in managed mountain forests may reach 33.9 m<sup>3</sup>/ha on average (*e.g.* Paillet et al. 2015). Furthermore, the peak of saproxylic organism diversity is rather observed in mixed mountain forests, which have 30-40 m<sup>3</sup>/ha of deadwood (Müller & Bütler, 2010).

Decomposers, detritivores and plant symbionts were not found to be more strongly influenced by forest variables than other trophic levels. Rather than for whole trophic levels, forest features are especially important for certain trophic groups.

## **Importance and effects of forest quantity features on soil trophic groups**

The group of ectomycorrhizal fungi is one of the trophic groups most influenced by forest variables in our results (Fig. 2). Indeed, ectomycorrhizal fungi are directly related to trees since ectomycorrhizae are primarily an association between fungi and tree species (Genre et al., 2020). Our results are in agreement with another study which shows that trees, hosts of ectomycorrhizae, seem to explain a significant part of the diversity of these fungi at large scales (van der Linde et al., 2018).

The importance of forest variables for the other trophic groups could be due to a less direct linkage than for ectomycorrhizal fungi and in particular be more related to light availability in the understorey. The importance of the quantity of light diffusing through the canopy could explain the fact that soil biodiversity is more important for low basal areas. Indeed, when the density of trees is high, the canopy regulates and strongly reduces the amount of light reaching the forest floor (Kovács et al., 2017). The understorey vegetation is limited by this lack of light and the understorey plant diversity is low due to the competitive exclusion of the trees. On the contrary, when the area occupied by trees is low, canopy is sparse and the availability of light energy increases, allowing an increase in plant diversity which is favourable to the diversity of soil organisms (Paillet et al., 2010; Scherber et al., 2010). A similar phenomenon occurs in stands where conifers dominate indeed stands dominated by conifers are usually subalpine forest which present low basal areas (Laughlin et al., 2005).

In general, we found that the diversity of trophic groups tended to decrease with increasing basal area as shown in other studies (Bouget et al., 2014; Tedersoo et al., 2016). Arbuscular mycorrhizal fungi diversity could be a good example of this indirect link with the amount of light transmitted to the understorey. Arbuscular mycorrhizal fungi do not form associations with tree species but rather with other plant life forms (Genre et al., 2020). We have shown that the diversity of arbuscular mycorrhizal fungi is higher for low basal area and high C/N ratios of tree leaves which is a characteristic of conifers. The higher understorey plant diversity associated with these forest conditions could explain the response of the arbuscular mycorrhizal fungi group (Gao et al., 2014; Laughlin et al., 2005; Öpik et al., 2008).

The importance of forest variables for trophic groups goes beyond the trophic levels that feed or live in direct association with plants. We showed that forest variables also explain well the diversity of fungivorous mites, predatory insects and parasitic protists. It can be assumed that there is a bottom-up control of groups in the different trophic levels of the soil network, conditioned by the nutrient resources and habitats provided by the forest. It can be seen, for

example, that forest variables have an effect on different groups of fungi and that fungivore mites tend to respond in the same way as fungi (Table 1).

### **Importance and effects of forest diversity features on soil trophic groups**

The variables describing forest diversity explain the variation in the diversity of about ten soil trophic groups. As we assumed, the diversity of trophic groups increases when the heterogeneity of the forest structure and composition increases which is in agreement with the heterogeneity-diversity hypothesis and other studies (*e.g.* Penone et al., 2019). Heterogeneity of forest structure could favour a multitude of microhabitats and microclimatic conditions that could be related to a high ecological niche diversity (Frey et al., 2016; Kovács et al., 2017; Lindenmayer et al., 2022). Due to a complementarity effect and especially niche partitioning, the diversity of organisms could be increased due to this heterogeneity of the forest structure. Two heterogeneity variables often show a significant positive relationship with the trophic groups studied, the Gini coefficient of tree diameters and deadwood diversity.

Studies on the relationship between tree diameters and soil biodiversity are rare. Janssen et al. (2018) do not find for instance, a link between heterogeneity of tree diameters, used as an indicator of forest maturity, and springtail diversity. However, some studies show that the biodiversity could be higher in stands with heterogeneous tree diameters during regeneration, late successional stages or in unmanaged forests, which conditions correspond to high Gini coefficient values (Hilmers et al., 2018; Pach & Podlaski, 2015; Paillet et al., 2015).

Deadwood is a source of varied micro-habitats that support a high level of biodiversity (Seibold et al., 2016). As with living trees, deadwood from different species may contain recalcitrant compounds that require special decomposers and detritivores adaptations, to be decompose. Thus, different communities succeed one another on deadwood pieces to degrade lignins and cellulose down to simplest molecules (Kraus et al., 2013). Biodiversity associated with deadwood on the ground (stumps or logs) is different from that associated with standing dead trees (Lassauce et al. 2011). For example, the higher moisture content of deadwood on the ground facilitates the access to OM to decomposers, unlike standing dead trees (Kraus et al., 2013; Parisi et al., 2018).

Tree diversity seems to be of little importance for the diversity of soil trophic groups. Penone et al. (2019) find a significant and negative effect of tree diversity on belowground biodiversity while Tinya et al. (2021) do not observe an effect of tree diversity on fungal groups. The positive effect we show could again be the result of complementarity effects. For instance, Gillespie et

al. (2021) show the importance of the diversity of leaf and root traits associated with tree species diversity for soil functioning.

### **Limitations and perspectives**

The relevance of the scale of study chosen to investigate soil trophic groups is questionable. We studied the links between forest structure and composition with soil organism diversity at a very local level on 150m<sup>2</sup> plots. The influence of forest characteristics could also take place at a larger scale such as the forest patch. For example, different tree species within a few metres of the plot could change the nature of the litter that reaches the surrounding soil organisms through wind or slope action. The influence of deadwood on saproxylic biodiversity could also depend on the dispersal capacity of organism and thus determine the scale at which deadwood should be studied (Haeler et al., 2021).

Other forest variables could be considered. In particular, tree species explain more about the diversity of soil organisms than tree diversity *per se* (Tedersoo et al., 2016). This may explain the low number of trophic groups that respond to the diversity of living trees in our study (Table 1). Furthermore, the altitudinal gradient (highly correlated with temperature, Annex 2) also corresponds to plant compositions that range from mixed forests at low elevations to coniferous forests at higher elevations. The change in forest composition with altitude seems to influence soil communities (Ganault et al., 2021) and would be an aspect to explore.

Finally, the forest variables studied could also influence other aspects of biodiversity such as the abundance of organisms or the composition of communities. Indeed, some studies show that tree species composition, basal area, tree biomass and diversity, or bush cover density structure soil communities in forest ecosystems (Chalmandrier et al., 2019; Rota et al., 2020; Tinya et al., 2021). More than the number of taxa present in the forest, taxa adapted to forest-specific conditions (*e.g.* light availability, recalcitrant litter compounds) could be better described by forest composition and structure.

To conclude, we have shown the importance of forest structure and composition for the study of soil trophic groups. Heterogeneity of forest conditions is associated with high soil trophic biodiversity. This study contributes to a better understanding of the factors that structure soil biodiversity. In the face of climate change, long-term observatory such as the Orchamp project are valuable for understanding the influence of climate on ecosystems and could allow long-term monitoring of organisms and soil functioning *in natura*.

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# Annexes

**Annexe 1 :** Minimum, maximum, medians, means and standard deviations (SD) of the data used as explanatory variables with the following abbreviations : Tree diversity = Hill number with  $q=1$  ; Community Weighted Mean (CWM) of C/N ratio of trees leaves ; Freezing Degree Days (FDD) ; Climatic Water Stress (CWS) ; Soil Organic Mater (OM). Values round up to the hundredth.

	Forest features						Climate			Soil		
	Basal area (m <sup>2</sup> /ha)	Deadwood volume (m <sup>3</sup> /ha)	CWM Leaves C/N of trees	Gini of basal area	Deadwood diversity	Tree diversity	Temperature	FDD	CWS	Soil pH	Soil OM	Soil C/N
Minimum	0	0	0	0	0	0	4,05	-176,67	-609,86	3,71	8,64	10,84
Maximum	178,56	57,06	81,91	0,90	10	4,27	13,28	-25,02	-160,77	8,07	82,38	37,38
Median	34,71	1,41	33,52	0,30	1	1,28	7,59	-55,54	-400,75	6,06	24,42	18,44
Mean	37,13	5,67	39,33	0,32	1,42	1,46	7,66	-62,96	-393,19	5,88	28,62	19,05
SD	28,28	9,67	24,75	0,23	1,60	0,79	2,19	31,20	112,44	1,14	15,37	4,98

**Annexe 2 :** Pearson correlation coefficient of the studied environmental variables.

	Forest Quantity Features			Forest Diversity Features			Climate				Soil		
	Basal area	Deadwood volume	CWM trees C/N	Gini of basal area	Deadwood diversity	Tree species diversity	Elevation	Temperature	FDD	CWS	pH	OM	Soil C/N
Basal area	1,00												
Deadwood volume	0,34	1,00											
CWM trees C/N	0,40	0,28	1,00										
Gini of basal area	0,28	0,10	0,05	1,00									
Deadwood diversity	0,39	0,61	0,31	0,18	1,00								
Tree species diversity	0,33	0,13	0,25	0,50	0,29	1,00							
Elevation	0,10	0,05	0,19	-0,34	0,04	-0,27	1,00						
Temperature	-0,11	-0,07	-0,23	0,34	-0,05	0,26	<b>-0,98</b>	1,00					
FDD	-0,10	-0,11	-0,06	-0,18	-0,23	-0,11	-0,18	0,09	1,00				
CWS	0,34	0,20	0,09	0,10	0,09	0,16	0,24	-0,21	-0,30	1,00			
pH	-0,29	-0,22	-0,31	0,05	-0,10	0,02	-0,40	0,42	0,10	-0,34	1,00		
OM	0,15	0,14	0,11	-0,10	0,04	-0,12	0,30	-0,31	0,05	0,00	-0,05	1,00	
Soil C/N	0,26	0,24	0,45	-0,14	0,29	-0,02	0,33	-0,33	-0,16	-0,15	-0,24	0,28	1,00

# Abstracts and key-words

## Résumé :

Les forêts de montagnes ainsi que leur fonctionnement sont particulièrement menacés par le changement climatique. Acteurs de la décomposition, les organismes du sol ont une place centrale dans le fonctionnement des écosystèmes forestiers mais cette biodiversité du sol a longtemps été assez mal connue. L'analyse d'ADN environnementale permet aujourd'hui des études multitaxonomiques et à de larges échelles. Nous avons étudié l'influence de variables climatiques, édaphiques, de structure et de composition forestière sur la diversité de 37 groupes trophiques du sol déterminés à partir d'ADNe et le long de 16 gradients altitudinaux répartis du nord au sud des Alpes françaises et appartenant au projet Orchamp. Nos résultats montrent que la structure et la composition forestière sont moins importantes que les variables abiotiques mais compte pour expliquer la diversité des groupes trophiques du sol. De plus, la diversité des conditions forestières favorise la diversité des organismes du sol étudiés. Cette étude participe à améliorer notre compréhension de l'importance relative des facteurs abiotiques par rapport aux conditions forestières dans le contrôle de la biodiversité du sol ce qui pourrait permettre aux gestionnaires d'espaces naturels de mettre en place des moyens d'action pour conserver la biodiversité ou permettre de développer des modèles prédictifs des impacts du changement climatique.

**Mots-clés :** Forêts ; groupes trophiques du sol ; ADN environnemental ; Structure forestière ; Composition forestière

## Abstract :

Mountain forests and their functioning are particularly threatened by climate change. Soil organisms, which are involved in decomposition, play a central role in the functioning of forest ecosystems, but this soil biodiversity has long been poorly understood. Environmental DNA analysis now allows multitaxonomic studies on large scales. We studied the influence of climatic, edaphic, forest structure and composition variables on the diversity of 37 soil trophic groups determined from eDNA along 16 altitudinal gradients distributed from north to south of the French Alps and belonging to the Orchamp project. Our results show that forest structure and composition are less important than abiotic variables but are important in explaining the diversity of soil trophic groups. Moreover, the diversity of forest conditions favours the diversity of soil organisms studied. This study contributes to our understanding of the relative importance of abiotic factors versus forest conditions in the control of soil biodiversity, which could participate to the work of natural area managers to implement means of action to conserve biodiversity or to develop predictive models of climate change impacts.

**Key-words :** Forests; Soil trophic groups; Environmental DNA; Forest structure; Forest composition