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# **Response of understory plant communities and traits to past land use and coniferous plantation**

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## Abstract

**Questions:** How did past land use and conifer plantation affect understory plant communities? What plant traits explain understory vegetation response to agricultural past land use and coniferous plantation?

**Location:** Forest of Orléans (50 000 ha), Loiret, northern central France.

**Methods:** Canopy cover, herb layer composition, litter and soil properties were measured in 80 100 m<sup>2</sup> plots sampled in plantations of *Pinus sylvestris* or *P. nigra* vs. natural regenerations of *Quercus petraea* and *Q. robur* along a gradient of forest continuity (using historical maps and aerial photos from 1840, 1949 and 2006). We related 15 plant traits to past land use and tree species using RLQ and fourth-corner analyses.

**Results:** The magnitude of past land use effect largely exceeded that of coniferous plantation. Post-agricultural forests, even 150 years after afforestation, differed from ancient forests in soil properties (thinner humus layer, higher phosphorus content, higher pH and lower C/N ratio) and plant traits. Ancient forests hosted more forest core species, stress-tolerant, competitive species and bryophytes, but fewer ruderals, annuals/biennials, shrubs and trees and nutrient-demanding species, and had lower specific leaf area and higher leaf dry matter content. Conifer plantations had a thicker humus layer, lower pH and higher C/N ratio than deciduous forests, and hosted more light-demanding species but less forest core species. Importantly, differences in soil and plant traits between conifer plantations and naturally regenerated deciduous stands were discernible in both recent and ancient forests, indicating that tree species lastingly influence ecosystem functioning.

**Conclusions:** Past land use and coniferous plantation resulted in different understory plant communities and traits under pine plantations and naturally regenerated oak stands. These differences were driven by soil acidification, litter decomposition rate, light conditions, canopy development, soil disturbance, stand management, and dispersal and recruitment limitations. Coniferous plantations slowed down the recovery of post-agricultural forests to ancient deciduous forest conditions. Applying natural regeneration and favouring native deciduous tree species thus helps recreate or maintain ancient forest plant communities.

**Keywords:** ancient forest, coniferous plantation, functional trait, land use history, tree species substitution, restoration.

**Nomenclature:** French BDTFX flora nomenclature and European BDNBE bryophyte nomenclature (<http://www.tela-botanica.org>).

## Introduction

Two major changes have occurred in European forest landscapes over the last 250 years. First, forest cover has globally increased in many European countries since the Industrial Revolution (Mather et al. 1999) due to rural exodus and agricultural land set-aside (Cramer et al. 2008). In France and several other European countries, many former agricultural lowlands have been planted with *Pinus* and *Picea* species (see <http://inventaire-forestier.ign.fr/spip/spip.php?rubrique80> and Leuschner et al. (2013)). Second, large areas of deciduous forests have been converted to coniferous forests in western and central Europe since the beginning of the 20<sup>th</sup> century, to develop more profitable industrial forestry (Wulf & Heinken 2008). There is still intense debate on whether plantation forests help to maintain biodiversity or instead degrade it (Bremer & Farley 2010). Some authors consider that plantations can accelerate the regeneration of native understory species and thus help conserve biodiversity (Boothroyd-Roberts et al. 2013), while others have shown no effect or a decrease in the number or the diversity of forest species (Aubin et al. 2008; Tullus et al. 2013). The value of plantations for biodiversity actually depends on several factors that are not always easy to disentangle: (i) tree species identity (ii) the nature of the earlier land cover (iii) proximity to native vegetation source, (iv) plantation silviculture and (v) soil conditions.

The role of tree species on understory plant communities is largely documented (Barbier et al. 2008). The overstory directly affects the understory through its impact on light availability (Wulf & Naaf 2009) and by competition for water and nutrients (Barbier et al. 2009), especially on acidic sites (Härdtle et al. 2003). Canopy composition also indirectly affects understory through litter quality and decomposability (Hagen-Thorn et al. 2004), which influences topsoil nutrient availability and acidity (Augusto et al. 2015), the soil fauna (Reich et al. 2005) and the recruitment and growing conditions of seeds (Baeten et al. 2009a). Understory plant composition differs between coniferous and deciduous stands and can depend on the deciduous tree species being compared (Barbier et al. 2008).

Past land use lastingly influences forest soil properties and understory vegetation, which gave rise to the concept of ancient forest (AF) and ancient forest species (AFS) (Hermý & Verheyen 2007). Forest soils on former arable fields are mostly differentiated from ancient forest by higher soil pH and phosphorus content, and lower nitrogen and carbon content (Sciama et al. 2009; De Schrijver et al. 2012). The association between forest continuity and ancient forest species is mainly related to their low dispersal capacity and low recruitment success in post-agricultural forests (Hermý & Verheyen 2007); recruitment of ancient forest species in post-agricultural forests is partly determined by interspecific competition, soil characteristics and light availability (Baeten et al. 2009b).

Understory-overstory relationships have generally been investigated without considering past land use (Barbier et al. 2008; Kooijman 2010). Although several studies on land use legacies have investigated the influence of tree species or regeneration mode (Aubin et al. 2008; Tullus et al. 2013; Thomaes et al. 2014), these have several limitations: (i) they did not include ancient forests as a control in their

sampling design, (ii) they explored a very short gradient of age since afforestation (<40 years) and (iii) they rarely compared deciduous and coniferous species. To our knowledge, only one study investigated the two gradients (Wulf & Heinken 2008) and found that deciduous stands did not guarantee higher richness of forest specialists, and underlined the role of former land use on the colonisation of forest species. There is still a need to explore the combined effects of past land use and tree species in more detail (Thomaes et al. 2012), in particular how conifer introduction can influence the recovery rate of understory plant communities to ancient forest conditions, using ancient coniferous forests as a control. Our aim was to investigate to what extent conifer plantations modified the composition and the traits of understory plant communities in recent and ancient forests compared with similar sites with deciduous tree species, addressing the three following questions:

(1) How did past land use and tree species affect understory plant communities and their traits and which of the two drivers was more influential?

(2) How did understory plant succession differ between conifers and deciduous forests in post-agricultural stands?

(3) What plant traits (ecological performance, resource requirement, persistence, regeneration, or dispersion traits) explain understory plant response to past land use and conifer plantation?

We assumed that understory communities were close in deciduous and coniferous post-agricultural forests then diverged with increasing time since afforestation, due to several ecological filters: soil acidification and litter accumulation, more pronounced in coniferous stands (Augusto et al. 2015), subcanopy layer development, greater and faster in naturally regenerated deciduous stands, canopy cover conditions, more open in pine forests (Augusto et al. 2003) and soil disturbance, more frequent in plantations (Boch et al. 2013). We expected traits related to ecological performance, resource requirement, persistence, regeneration and dispersion were linked to forest continuity (Verheyen et al. 2003), and traits related to ecological performance, resource requirement and regeneration were associated with tree species (Barbier et al. 2008), but which of the two gradients was more influential on plant communities and traits was difficult to predict. Following Wulf and Heiken (2008), we also hypothesised that migration of forest core species [*sensu* Pellissier et al. (2013)] into recent coniferous forests would be delayed compared with deciduous forests, due to both dispersal and recruitment limitations.

## Methods

### Study area, land use and forest management history

The study was conducted in the Forest of Orléans (48°00'28" N, 2°09'39" E), which contains a central block of ancient forest covering about 35,000 ha (Fig. 1). Elevation ranges from 107 to 174 m, climate is oceanic, and mean annual precipitation is about 700 mm. The study area was dominated by oak (*Q.*

*petraea* and *Q. robur*), pine (*Pinus nigra* subsp. *laricio* var. *corsicana* and *P. sylvestris*) and mixed oak-pine forests.

Forest cover reconstruction was based on two sources: historical maps of 1840 (Etat-Major map) and aerial photographs taken in 1949. Forest patches in 1840 were vectorised following a precise protocol (Favre et al. 2013) and then georeferenced with ArcGIS version 10.0 using about one reference point per km<sup>2</sup>. Land use in 1949 was checked on aerial photos. Current forest cover maps dating from 2006 were provided by the French National Forest Inventory ([www.inventaire-forestier.ign.fr](http://www.inventaire-forestier.ign.fr)).

Three forest age classes were defined in current forests: ancient forests (AF) (patches present in 1840, 1949 and 2006), intermediate-age forests (IF) (patches absent in 1840, present in 1949 and 2006), and recent forests (RF) (patches absent in 1840 and 1949, present in 2006). In the following, the term "post-agricultural forests" includes intermediate-age forests and recent forests.

These maps and other documents on State forest management history showed that the study area had undergone two major changes in land use and forest management since the beginning of the 19<sup>th</sup> century. First, a 23% net increase in forest area occurred between 1840 (59,300 ha) and 2006 (73,200 ha), the resultant of a gain (21,700 ha) and a loss (−7,800 ha), ancient forest thus covering 51,500 ha (Fig. 1). Second, conifers were extensively planted on former agricultural lands but also on former deciduous forests. Conifers currently represent 40% of forest cover, compared with 6% of the total in 1870, and were absent in 1840. For historical reasons, we cannot separate the effects of regeneration mode from the effect of overstory composition, because Corsican and Scotch pines were planted, whereas most of the time sessile and pedunculate oaks were naturally regenerated.

## Sampling design

We selected 80 sampling points crossing two types of dominant tree species composition (TSC): coniferous, mainly *P. sylvestris* and *Pinus nigra* subsp. *laricio* var. *corsicana*, noted CON vs. deciduous, mainly *Quercus petraea* - *Q. robur*, noted DEC; and the three classes of forest temporal continuity (FTC): ancient, intermediate-age and recent forests (Table 1). To reduce stand variability, we selected forest stands that were between 40 and 100 years old and only considered "pure stands" with more than 70% of the total basal area of either coniferous or deciduous species.

## Floristic survey, humus form and soil data collection

At each of the 80 sampling points, the presence of understory vascular species (pteridophytes and phanerogams) and terricolous bryophytes below 2 m in height were recorded in one 10 × 10 m plot between June 9 and July 22, 2011. The vegetation belongs to the phytosociological sub-alliance of *Quercenion robori-petraeae* Rivas Mart. 1975. We also measured canopy cover of different strata and humus form, and analysed soil samples to measure pH H<sub>2</sub>O, organic carbon content (C<sub>org</sub>), total nitrogen content (N content), potentially available phosphorus content and other soil parameters at two depths (0-

5 cm and 15-20 cm). Appendix S1 gives a detailed presentation of forest stand and soil data protocol. Soil types were cambisols, dystric cambisols, planosols, entic podzols and podzols (WRB 2015). Topsoil texture was mostly sand or loamy sand with a predominantly clay horizon (silty clay, sandy clay or clay) appearing between 30 and 70 cm. Moderate waterlogging was observed between 24 and 68 cm. pH H<sub>2</sub>O ranged from 3.9 to 5.7 and humus form varied from mesomull to dysmoder according to the classification of Ponge et al. (2002).

## **Plant traits**

We investigated plant traits that were previously related to forest understory succession after land abandonment (Hermy & Verheyen 2007) and that could vary according to tree species (Barbier et al. 2008). We selected 15 species traits and ecological features, and classified them according to their ecological function (Table 2): ecological performance, resource requirement, persistence, regeneration and dispersal ability (Verheyen et al. 2003; Violle et al. 2007). Trait values were extracted from several databases (Catminat, Ecoplant, Ellenberg, LEDA, Bioflor and D<sup>3</sup>). Although plant features related to ecological performance are not functional traits, we use the term "plant traits" to simplify. Details on plant traits are provided in Appendix S2. Detailed hypotheses on the expected relationships between each trait and the two gradients we investigated are presented in Appendix S3.

## **Data analyses**

### ***Soil and stand variations among sites***

Preliminary analyses checked for soil and stand variations among sites (Appendix S1). To reduce the number of predictors in our analyses, we selected a short list of five environmental variables that are recognised to vary with forest continuity and tree species and reflected nutrient richness, light availability and past disturbance regime (Thomaes et al. 2012): humus index (litter effect on understory plants), pH H<sub>2</sub>O (nutrient balance between exchangeable H/Al and exchangeable cations), phosphorus content (legacy of former agricultural use) and light conditions: lower and upper canopy cover (CC 2–8 m and CC > 8 m).

### ***Plant communities***

To test how plant community composition varied with forest continuity and tree species, we first applied canonical correspondence analysis to the L-table after Hellinger transformation with forest continuity and tree species as explanatory variables. We conducted permutation tests to analyse the effects of the two factors and their interaction using 9999 permutations. We then performed a pCCA using forest continuity and tree species as constraints and humus index, pH H<sub>2</sub>O, log(P<sub>2</sub>O<sub>5</sub>+1), CC 2–8 m and CC > 8 m as conditions to test whether forest continuity and tree species effects persisted after controlling for resource gradient differences. We did not apply RLQ, a method to directly analyse trait-environment relationships (Kleyer et al. 2012), because this approach is not adapted to test the effect of a set of

variables after controlling for the effect of covariates (Jamil et al. 2013). Between-class RLQ is available (Dray et al. 2014), but impossible to apply when one aims at removing the effect of several variables, whereas partial canonical correspondence analysis (pCCA) is suited for this purpose (Borcard et al. 1992).

### ***Plant traits***

To analyse how species traits were influenced by forest continuity and tree species and which of the two gradients had a greater influence, we adopted the analysis framework proposed by Dray *et al.* (2014): we used RLQ to obtain a graphical display and related values of projections and fourth-corner analysis (Legendre et al. 1997) for testing statistical relationships between traits and environmental variables. RLQ was used to provide a simultaneous ordination and analyse the joint structure of three tables: R (environmental gradients: forest continuity, tree species, humus index, pH H<sub>2</sub>O, log(P<sub>2</sub>O<sub>5</sub>+1), CC 2–8 m and CC > 8 m), L (plant presence data) and Q (species traits).

A multivariate test using permutation method was applied to evaluate the global significance of the trait-environment relationships [see (Dray et al. 2014) and Appendix S4]. Using permutation method, we also tested the links between RLQ axes and traits or environmental variables to interpret the main pattern of variation and correlation (Dray et al. 2014). As the L-table contained presence-absence data only, categorical trait variation reflected change in proportion of each trait category. Fourth-corner was lastly applied to test the significance of bivariate associations between each trait, or each trait category, and each environmental variable. The link was measured by a Pearson correlation coefficient for two quantitative variables (trait and environmental variable), by a Pearson Chi<sup>2</sup> and G statistic for two qualitative variables and by a Pseudo-F and Pearson r for one quantitative variable and one qualitative variable. We used 9999 permutations in all randomization procedures, and the false discovery rate method (FDR) to adjust *P* values for multiple testing. See Appendix S4 for more details on RLQ and fourth-corner analysis.

Finally, to test whether migration of forest core species [*sensu* Pellissier et al. (2013)] into recent coniferous forests would be delayed compared with deciduous forests, we ran a two-way ANOVA to test the effects of forest continuity, tree species composition and their interaction on the number of forest core species at plot scale.

All statistical analyses were performed using *R* software (version 3.2.5, R Foundation for Statistical Computing, Vienna, AT); we used the package *ade4* for RLQ and fourth-corner analysis (Dray et al. 2014) and the package *vegan* for CCA and pCCA.

## **Results**

### **Plant communities**



The CCA applied to the floristic matrix composed of 80 sampling points and 197 species showed that 12.6% of the total variance was explained by forest continuity, tree species and their interaction. The projected inertias on the first three factorial axes were 43, 25 and 16%, respectively. Permutation tests showed that the first two factorial axes and main effects were highly significant ( $P < 0.001$ ) and the interaction was very significant ( $P = 0.004$ ). Ancient, intermediate-age and recent forests were ordered along the first axis and the second, orthogonal axis discriminated between oak and pine stands (Fig. 2). Community dissimilarity between oak and pine was present along the forest continuity gradient but was much higher in recent compared to ancient forest. Plant turn-over from ancient to recent forest was slightly higher for pine. A partial CCA using forest continuity and tree species as constraints and humus index, pH H<sub>2</sub>O, log(P<sub>2</sub>O<sub>5</sub>+1), CC 2–8 m and CC > 8 m as conditions indicated that 8.5% of the total variance was explained by forest continuity and tree species, while the other environmental variables explained 14.0% of the total variance. Permutation tests showed that the main effects and interaction were still significant (forest continuity:  $P = 0.001$ ; tree species:  $P = 0.002$  and interaction:  $P = 0.02$ ). Individual plant species response to past land use and conifer plantation are presented in Appendices S5 and S6.

### Plant traits

RLQ analysis showed that plant traits were significantly related to forest continuity, tree species and environmental variables (permutation tests for *model 2* and *model 4*:  $P < 0.0001$ , see Appendix S4 for description of *models 2* and *4*). The first two axes of the RLQ accounted for 95% of the total inertia (87% and 8% respectively). The variance of the environmental scores (R-table) was almost entirely preserved on the first two axes (98%). The variance of the trait scores (Q-table) was also well preserved (40% on the first axis and 58% on the first two axes). The first ordination axis of the RLQ was associated with forest continuity and contrasted ancient forests (negative values) and recent forests (positive values), intermediate-age forests being roughly in the middle (Fig. 3a and Table 3). The second axis was related to tree species, and differentiated deciduous stands (positive values) from coniferous ones (negative values, Table 3). Humus index and canopy cover above 8 m were negatively associated with the first axis (higher values in ancient forest and lower values in recent forest), while pH H<sub>2</sub>O and P content displayed the opposite trend (lower values in ancient forest and higher values in recent forest; Fig. 3a and Table 3). Canopy cover 2–8 m was positively associated with the second ordination axis, indicating higher subcanopy cover in deciduous stands (Fig. 3a and Table 3). Since the first two RLQ axes were respectively closely related to forest continuity and tree species, the correlations between these gradients and plant traits were easy to infer. All the traits were correlated with at least one RLQ axis (Fig. 3b and Table 4). However, the traits or trait categories that correlated with the first axis (forest continuity) differed from the traits that correlated with the second axis (tree species): only seed length and zoochory were significantly correlated with the first two axes (Table 4).

Forest core species, leaf dry matter content and bryophytes were highly, negatively correlated with the first axis ( $P < 0.001$ ), indicating increasing values from recent to ancient forest (Table 4). C-coordinate, S-coordinate and seed length were also negatively correlated but to a lower extent ( $P < 0.01$  or  $0.05$ ). Conversely, R-coordinate, indicator value for soil pH and soil nitrogen (N), specific leaf area and annuals/biennials were highly positively correlated with the first axis, indicating decreasing values from recent to ancient forest. Perennial herbs, species reproducing by seed and vegetatively (sv.vvs), barochory and zoochory were also positively correlated with the first axis ( $P < 0.01$  or  $P < 0.05$ ). Bivariate associations between categories of forest continuity and species traits indicated that peripheral species and shrubs/trees were associated with recent forests, while larger seeds and earlier end-of-flowering characterised intermediate forests (Table 5).

Seed weight and length, myrmecochory and zoochory were positively associated with the second axis ( $P < 0.01$ ) with higher values in deciduous forests (Table 4). Conversely, indicator value for light, start and end of flowering, anemochory and seed bank longevity index were negatively associated with the second axis ( $P < 0.01$ ), meaning that these traits had higher values in coniferous stands. In addition, proportion of forest core species was higher in deciduous forests (Table 5), proportion of annuals and biennials was particularly high in recent pine plantations (Fig. 3b), and several species were located in this bottom right corner of the first RLQ factorial map (Appendix S7). We noted that the associations between the second axis of RLQ and traits (Table 4) and bivariate associations between tree species and traits (Table 5) were not consistent: only forest core species and indicator value for L were significantly related to tree species in Table 5, meaning that the associations between the second RLQ axis and traits should be interpreted with caution.

The *ad-hoc* two-way ANOVA applied to forest core species richness revealed that the effects of forest continuity and tree species were significant ( $P < 0.001$  and  $P < 0.01$  respectively) but not the interaction: species richness increased from recent to ancient forest and was higher under oak (Fig. 4).

## Discussion

Our results showed that forest continuity and tree species acted as ecological filters on the taxonomic and functional composition of forest understory plant communities. Plant trait composition varied along the forest continuity and tree species gradients according to ecological performance, resource requirement, persistence, regeneration and dispersal ability. As past land use was correlated to the first RLQ axis (87% of total inertia) while tree species was correlated to the second one (8%), our results indicated that the magnitude of past land use effect on plant functional composition was globally stronger than the effect of tree species, in line with Kelemen *et al.* (2014).

### Role of past land use

Previous cultivation has long-lasting effects on the upper forest soil horizon, e.g. nitrogen and carbon depletion, phosphorus enrichment, and soil pH rise (Hermy & Verheyen 2007; Berthrong *et al.* 2009;

Brudvig et al. 2013). Overall, land use legacies on topsoil chemical properties are related to three processes: (i) soil acidification and base cation loss caused by nutrients being redistributed from soils to biomass in aggrading forest ecosystems (Jobbagy & Jackson 2003), (ii) lower rates of nitrification and higher nitrate leaching in the soil, which may result from increased soil acidity (Hermy & Verheyen 2007), and (iii) past amendment and fertiliser application (lime and manure) in former agricultural soils that have enriched soil P and base cation levels (Compton & Boone 2000). As expected, we found a pronounced agricultural legacy on understory plant communities and progressive recovery to ancient forest communities, as evidenced by the first axis of the CCA (Fig. 2). As resource gradients (nutrient and light) did not explain all the variability in plant community composition (see pCCA), this strongly suggests that factors other than local habitat quality constrained the establishment of forest understory plants in post-agricultural forests, *e.g.* time since disturbance, distance to propagule source and species dispersal capacity. After deforestation and subsequent cultivation, species typical of ancient forests are soon lost from the soil seed bank (Hermy & Verheyen 2007). After the abandonment of agriculture, the land is colonised by open habitat herbaceous plants, but ruderal species rapidly disappear and are replaced by competitive species (pioneer trees and shrubs) (Hermy & Verheyen 2007). In our study, plant C-S-R signature was characterised by a strong decrease in R-component and an increase in C and S-components from recent to ancient forest. The variation in R-component can be linked to ploughing and other forms of soil disturbance and the increase in C-axis to land abandonment and increased competitive exclusion (Hunt et al. 2004). Long after the reconstitution of a dominant tree layer, the plant communities still differ from ancient forests: many plants typical of ancient forests are lacking due to their limited dispersal capacity (Hermy & Verheyen 2007). In accordance with previous studies (Verheyen et al. 2003; Bergès et al. 2016), we showed that proportion of zoochory was slightly higher and seed length lower in recent forests (Table 4).

Plant communities in recent forests were characterised by a larger proportion of nutrient-demanding, ruderal and short-lived species, but also tree and shrub species. Some of the shrubs in our recent forests could be considered as relicts from previous stages in vegetation succession (Verheyen & Hermy 2001). The lower specific leaf area and higher leaf dry matter content detected in ancient forests confirms that species in environments with resource stress displayed these functional features compared with resource-rich environments (higher soil pH and N and P availability), such as recent forests (Cornelissen et al. 2003). Analogous responses of specific leaf area and leaf dry matter content to land use change were noted in grasslands, where abandonment of land tended to favour species with reduced acquisition capacities and increased nutrient conservation efficiency (Garnier et al. 2007).

### **Role of tree species composition**

Many conifer species, but not all (Barbier et al. 2008), tend to acidify soils more than deciduous trees through several processes (Verstraeten et al. 2013; Augusto et al. 2015). Although we detected significant differences in pH, C/N and phosphorus content between deciduous and coniferous stands

(Table S1 and Fig. S1), the fourth-corner analysis did not detect any significant associations between plant resource requirements (indicator values for pH and N) and tree species composition (Tables 4 and 5). This may be because RLQ is simply an ordination of the fourth-corner statistics and so does not build a truly multi-trait multi-environment model in the sense of regression analysis, *i.e.* is unable to reveal additive effects (Jamil et al. 2013). In our case, variations in soil properties were much stronger along the forest continuity gradient than between deciduous and coniferous forests: indeed, tree species effect was significant only after correcting for forest continuity effect (data not shown). Plant functional composition differed between oak and pine mainly for shade tolerance (Table 5), in line with a less dense canopy cover between 2 and 8 m under pine (Fig. S1) and the highest indicator value for light detected for the understory vegetation under *P. sylvestris* among six tree species (Augusto et al. 2003). The higher proportion of anemochory and lighter and shorter seeds in pine plantations (Table 4) could also be explained by their more open subcanopy, which could facilitate seed dispersal by wind. A higher proportion of vernal plants, which must bloom before canopy leaf expansion, could explain the earlier period of flowering observed in oak (Table 4).

### **Role of regeneration mode and forest management**

Regardless of tree species, site preparation before plantation implies more intense forest floor and topsoil disturbances (*e.g.* ploughing and soil scarification) compared with naturally regenerated deciduous stands (Balandier et al. 2006). This could explain why recent pine plantations exhibited higher proportions of ruderal and wind-dispersed species (Boch et al. 2013). Weed control by mechanical or herbicide release treatments can also deeply modify plant communities and promote the establishment of species adapted to disturbance (Balandier et al. 2006). Pine plantations in post-agricultural forests had a low proportion of ant-dispersed species, which can be explained by their reduced colonisation capacity after removal from a site (Aubin et al. 2008), and hosted plants with a higher seed bank longevity (Table 4), which is a strategy to survive in case of frequent site disturbances. Conserving a substantial part of the pre-existing plant communities in plantation forests thus has a positive impact on plant diversity and vegetation recovery (Hartley 2002). Likewise, the silviculture applied to the stand plays a role because it modifies light transmittance to the ground and causes regular soil disturbance due to logging machines (Verstraeten et al. 2013). Moreover, shrub and subcanopy strata can be controlled during thinnings at the thicket, sapling and pole stages of the stand and could mitigate soil acidification in pine plantations (Van Nevel et al. 2014). Silviculture in conifer plantations prevents natural development of a dense tree subcanopy, as shown by the much lower canopy cover between 2 and 8 m under pine (Fig. 3a and Fig. S1). On the other hand, intense litter and topsoil disturbances could also occur in oak to favour natural deciduous regeneration in actively managed stands. Consequently, our results cannot be interpreted as pure tree effects, but rather a combination of tree species effect and their associated management (Thomaes et al. 2012).

## **Understory succession differed between pine plantations and naturally regenerated oak stands**

Soil chemical parameters in recent forests under oak and pine were very close and confirmed that pre-afforestation site conditions were comparable (Fig. S1). Litter accumulation was higher and litter degradation lower under pine, and these differences increased with time after pine plantation (Fig. S1). In contradiction with our initial hypothesis and with the patterns observed on soil, CCA indicated higher composition similarity between deciduous and coniferous ancient forests than between corresponding recent forests (Fig. 2), *i.e.* a convergence between pine and oak plant succession with increasing forest continuity. To explain the higher dissimilarity in recent forests, we noted that recent pine plantations displayed a higher proportion of short-lived species (Fig. 3b). Floristic difference between pine plantations and deciduous stands in recent forests (Fig. 2) may be related to a higher maintenance of ruderal and nutrient-demanding species due to more intense initial site disturbances combined with lower canopy cover under pine (Thomaes et al. 2014). Then, the effect of the disturbance tends to dissipate progressively after the stand-initiating disturbance (Fourrier et al. 2015), and other ecological filters take over: soil acidification and phosphorus diminution reduced the pool of ruderal and nutrient-demanding species and subcanopy development reduced light-loving competitors and promoted the establishment of shade-tolerant forest herbs (De Keersmaecker et al. 2011). Increasing soil acidification and litter accumulation in intermediate and ancient pine plantations limited the colonisation of acid-intolerant forest species, while promoting acid-tolerant forest species (Thomaes et al. 2013), which partly explains why plant dissimilarity between pines and oaks persisted in intermediate and ancient forests (Fig. 2).

In accordance with our initial hypothesis, oak forests hosted a slightly higher proportion of forest core species (Table 5) and a significantly higher number of forest core species (Fig. 4), which suggests that migration or recruitment of forest specialists were delayed in pine plantations on farmlands. This contrasts with the comparably efficient migration of herbaceous forest specialists into recent coniferous and deciduous stands observed by Wulf and Heinken (2008). Our result is consistent with previous experiments that underlined that acidifying tree species could severely limit germination and survival of ancient forest species (Thomaes et al. 2014).

Overall, understory succession in post-agricultural stands varied between pine plantations and naturally-regenerated oak stands, and differences persisted in ancient forests. In line with Fourrier *et al.* (2015), we attributed these differences to the intensity of the initial disturbance, and then to tree species and contrasting stand management.

## **Conclusion**

Plant communities and traits in pine plantations under recent and ancient forests differed from similar sites with pedunculate and sessile oaks. Past land use and tree species substitution had combined effects on topsoil chemical properties, plant community and plant traits, and the impacts of past land use were globally stronger than those of conifer plantation. This hierarchy has to be validated in other ecological

contexts and tree species. Understory plant succession differed between pine plantations and naturally-regenerated oak stands, under the influence of several ecological drivers: soil disturbance and understory vegetation removal at the beginning, and then tree species and stand management during stand development, where soil acidification, litter accumulation, canopy cover and dispersal and recruitment limitations play a major role. Our study underlines the slow process of recovery of recent forests towards ancient forest plant communities. In our study area, conifer plantation resulted in a long-lasting distinct taxonomic and functional trait composition. In ancient deciduous forests, we therefore advise applying natural regeneration and favouring native deciduous tree species to maintain or restore ancient forest plant communities.

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## **Supporting Information**

Additional supporting information may be found in the online version of this article.

Appendix S1. Detailed analysis of humus form, soil and stand variations among sites

Appendix S2. Plant trait data

Appendix S3. Set of hypotheses on the expected relationship between plant traits and ecological gradients

Appendix S4. Detailed methodology to analyse the relationship between plant traits and forest continuity and tree species

Appendix S5. Individual species response to forest continuity and tree species

Appendix S6. Detailed results of multiple logistic regression models applied to individual species

Appendix S7. Ordination of plant species in the first RLQ factorial map

**Table 1.** Definition of the six sampling units based on forest continuity (according to past land use maps of 1840 and aerial photographs taken in 1949) and tree species, and number of sampling points.

	Tree species composition		Total
	deciduous ( <i>Q. petraea</i> / <i>Q. robur</i> )	coniferous ( <i>Pinus sylvestris</i> )	
ancient forest (AF)	AF-DEC: deciduous ancient forest	AF-CON: plantation of pines between 1840 et 1949 on deciduous ancient forest	
	15	12	27
intermediate- age forest (IF)	IF-DEC: natural colonisation of deciduous tree species between 1840 and 1949 on former agricultural land	IF-CON: plantation of pines between 1840 and 1949 on former agricultural land	
	16	13	29
recent forest (RF)	RF-DEC: natural colonisation of deciduous tree species after 1949 on former agricultural land	RF-CON: plantation of pines after 1949 on former agricultural land	
	8	16	24
Total	39	41	80

**Table 2.** The 15 plant traits analysed by ecological function: trait name, data sources, type, trait category (continuous: C; ordinal: O; nominal: N) and code. Data sources: Catminat (<http://philippe.julve.pagesperso-orange.fr/catminat.htm>), Ecoplant (Gégout et al. 2005), Ellenberg indicator values (Ellenberg et al. 1992), LEDA (Kleyer et al. 2008), Biolflor (Kühn et al. 2004) and D<sup>3</sup> (Hintze et al. 2013).

Ecological function	Plant trait	Data source	Type	Category	Code
Ecological performance	Habitat preference: distance to edge	Pellissier et al. (2013)	N	Forest core species Forest peripheral species	HAB.core HAB.peripheral
	CSR strategy	Hunt et al. (2004)	C	C-coordinate S-coordinate R-coordinate	C.coord S.coord R.coord
	Ind. value for pH	Ecoplant	O	-	pH
	Ind. value for N	Ellenberg	O	-	N
Resource requirement	Ind. value for L	Ellenberg	O	-	L
	Specific leaf area	LEDA	C	-	SLA
	Leaf dry matter content	LEDA	C	-	LDMC
Regeneration	Plant life span + bryophytes	Catminat	N	annual or biennial	PLS.ann.bie
				perennial herb	PLS.per.herb
				shrub pr tree	PLS.shr.tree
				bryophyte	PLS.bryo
	Reproduction type	Biolflor	N	s: by seed only	REPRO.s
				ssv: mostly by seed, rarely vegetative	REPRO.ssv
				sv: by seed and vegetatively; vvs: mostly vegetative, rarely by seed	REPRO.sv.vvs
	Start of flowering	Catminat	C	-	beg.fl
Dispersion	Dispersal mode	Catminat	N	anemochory	DISP.anemo
				barochory	DISP.baro
				myrmecochory	DISP.myrmeco
				zoochory	DISP.zoo
	Seed weight (mg)	D <sup>3</sup> and Biolflor	C	log transformed	log.SW
	Seed length (mm)	D <sup>3</sup> and Biolflor	C	log transformed	log.SL
	Seed bank longevity index	LEDA	C	0: transient; 0.5: short-term persistent; 1: long-term persistent	SBLI

**Table 3.** Trait responses to forest continuity, tree species and other environmental variables using RLQ: fourth-corner tests (Pearson correlation coefficient  $r$ ) between the first two RLQ axes for plant traits (AxisQ1 and AxisQ2) and forest continuity, tree species and environmental variables.  $P$  values were adjusted for multiple comparisons using the FDR (false discovery rate) procedure. See also Fig. 3 for codes for environmental variables.

Code	Variable description	AxisQ1		AxisQ2	
		$r$	$P$ value	$r$	$P$ value
AF	Ancient forest	-0.309	***	-0.068	NS
IF	Intermediate-age forest	-0.141	***	0.082	*
RF	Recent forest	0.433	***	-0.016	NS
CON	Coniferous forest	0.025	NS	-0.152	***
DEC	Deciduous forest	-0.025	NS	0.152	***
Humus index	-	-0.430	***	-0.063	NS
pH H <sub>2</sub> O	-	0.434	***	0.006	NS
log(P <sub>2</sub> O <sub>5</sub> +1)	-	0.465	***	-0.048	NS
CC 2-8 m	Canopy cover 2-8 m	0.084	NS	0.148	***
CC > 8 m	Canopy cover > 8 m	-0.222	***	0.085	*

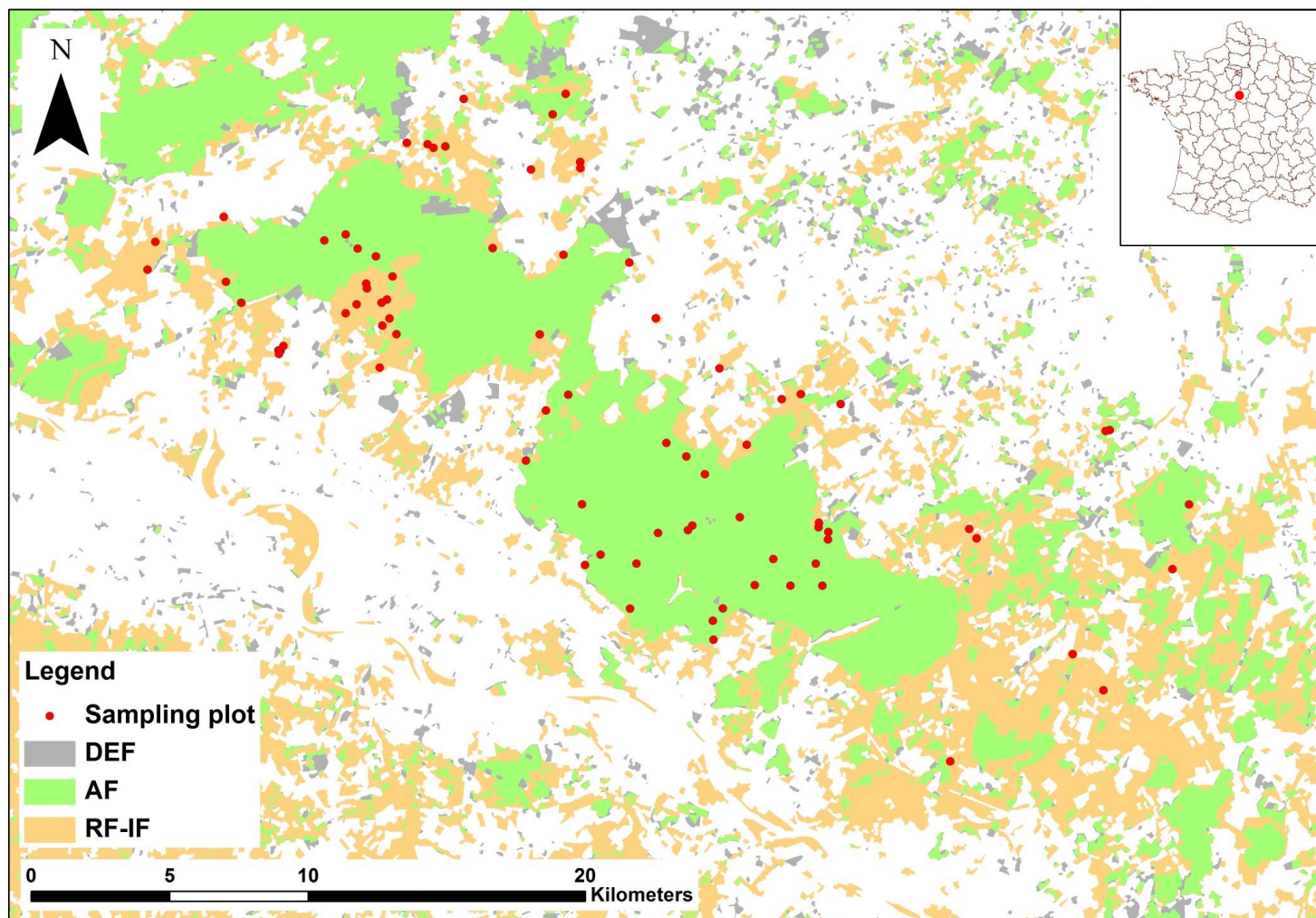
**Table 4.** Trait responses to forest continuity, tree species composition and environmental variables using RLQ: fourth-corner tests (Pearson correlation coefficient  $r$ ) between the first two RLQ axes for environmental gradients (AxisR1 and AxisR2) and plant traits.  $P$  values were adjusted for multiple comparisons using the FDR (false discovery rate) procedure. Codes for species traits are explained in Table 2.

		AxisR1		AxisR2	
		$r$	$P$ value	$r$	$P$ value
HAB.core	forest core species	-0.236	***	0.020	NS
HAB.peripheral	peripheral species	0.094	NS	0.057	NS
C.coord	coordinate for competition	-0.113	*	-0.018	NS
S.coord	coordinate for stress-tolerance	-0.148	**	0.064	NS
R.coord	coordinate for ruderality	0.266	***	-0.046	NS
pH	indicator value for soil pH	0.441	***	0.106	NS
N	indicator value for soil nitrogen	0.240	***	0.039	NS
L	indicator value for light	0.039	NS	-0.096	**
SLA	specific leaf area	0.225	***	0.014	NS
LDMC	leaf dry matter content	-0.232	***	0.028	NS
PLS.ann.bie	annual/biennial	0.247	***	-0.054	NS
PLS.bryo	bryophyte	-0.183	***	0.001	NS
PLS.per.herb	perennial herb	0.157	**	-0.026	NS
PLS.shr.tree	shrub/tree	-0.076	NS	0.044	NS
REPRO.s	reproduction by seed	0.038	NS	-0.027	NS
REPRO.ssv	reproduction mostly by seed, rarely vegetative	-0.058	NS	0.019	NS
REPRO.sv.vvs	reproduction by seed and vegetative	0.158	**	0.020	NS
beg.fl	start of flowering	-0.015	NS	-0.097	**
end.fl	end of flowering	0.045	NS	-0.124	***
DISP.anemo	anemochory	-0.026	NS	-0.122	***
DISP.baro	barochory	0.104	*	-0.053	NS
DISP.myrmeco	myrmecochory	0.009	NS	0.083	**
DISP.zoo	zoochory	0.108	*	0.095	**
log.SW	seed weight (log)	-0.009	NS	0.109	**
log.SL	seed length (log)	-0.107	*	0.084	**
SBLI	seed bank longevity index	-0.007	NS	-0.084	**

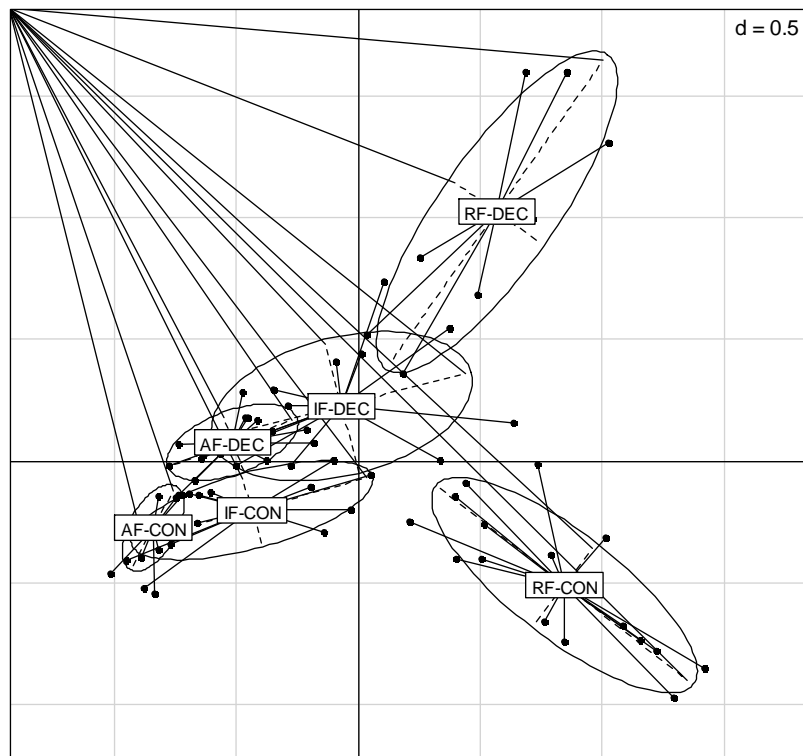


**Table 5.** Fourth-corner tests for bivariate associations between plant traits and environmental variables. The link is measured by a Pearson correlation coefficient for two quantitative variables (trait and environmental variable), by a Pearson  $\chi^2$  and  $G$  statistic for two qualitative variables and by a Pseudo-F and Pearson  $r$  for one quantitative variable and one qualitative variable (significant associations are in bold).  $P$  values were adjusted for multiple comparisons using the FDR (false discovery rate) procedure. Same legend as Tables 3 and 4.

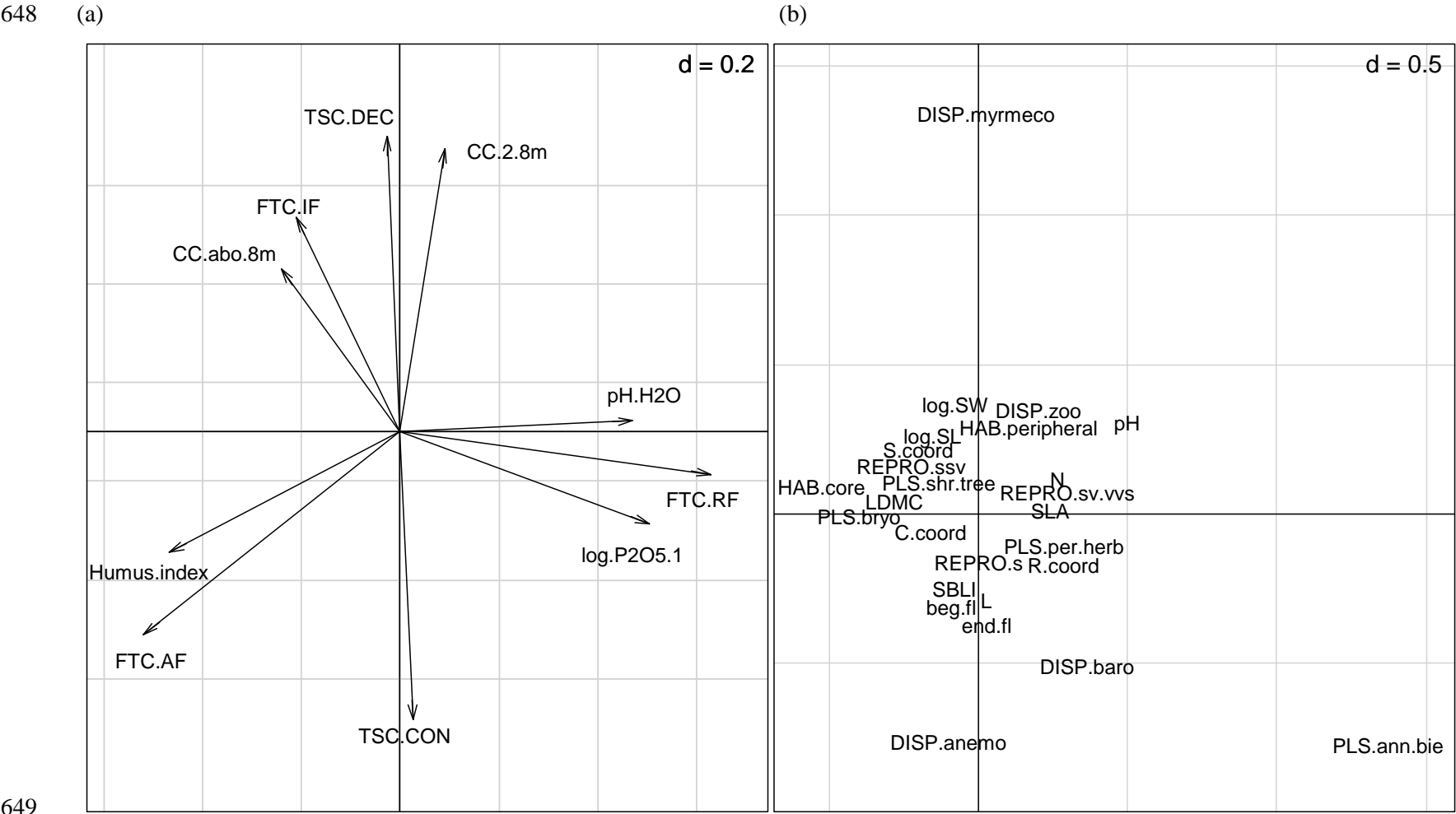
See file "Table 5.xlsx"



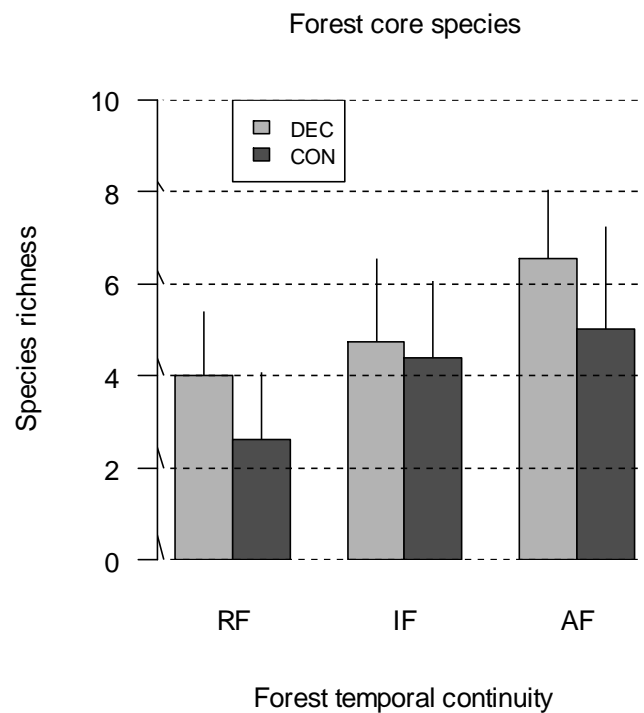
**Fig. 1.** Location of the 80 sampling points in the forest of Orléans (Loiret, France); AF: ancient forest (present in 1840 and 2006); RF/IF: recent or intermediate age forest (absent in 1840 and present after this date); DEF: deforestation (present in 1840 and absent in 2006).



**Fig. 2.** Biplot of the sampling points in the first factorial map of the CCA applied to the matrix of 80 sampling sites and 197 species using forest continuity and tree species as predictors. The value of  $d$  gives the grid size. The plots are grouped according to stand type: AF: ancient forests; IF: intermediate-age forests; RF: recent forests; tree species: DEC: deciduous; CON: coniferous. The ellipses comprised 67% of the points on the hypothesis that the scatter is a simple random sample following a bivariate normal distribution.



650 **Fig. 3.** Simultaneous ordination of (a) forest continuity, tree species and environmental variables and (b) species traits in the first RLQ factorial map. The  
651 value of  $d$  gives the grid size. Codes for environmental variables: AF: ancient forest; IF: intermediate-age forest; RF: recent forest; CON: coniferous; DEC:  
652 deciduous; CC >8 m: canopy cover above 8 m; CC 2-8 m: canopy cover 2–8 m (see text and Appendix S1 for details). See Table 2 for trait category code and  
653 Appendix S6 for plant name code.



654

655 **Fig. 4.** Species richness of forest core plants [*sensu* Pellissier et al. (2013)] as a function of forest  
656 continuity and tree species. The graph gives mean and standard deviation. AF: ancient forest; IF:  
657 intermediate-age forest; RF: recent forest; CON: coniferous; DEC: deciduous. Model results: forest  
658 continuity effect:  $P < 0.0001$ ; tree species effect:  $P = 0.008$ ; interaction:  $P = 0.39$ ; *adjusted-R*<sup>2</sup> = 0.324.