Fine scale species distribution changes in a mixed oak stand over two successive generations.

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Summary

- Large scale tree distribution changes have received considerable attention but underlying demo-genetics mechanisms are less documented. We used a diachronic approach to track species shifts in a mixed oak stand (*Quercus petraea/Q. robur*) at a fine spatiotemporal scale.
- We carried out a combined demographic, ecological and genetic monitoring before and after natural regeneration. Species assignment was made using SNP fingerprints and reproductive success estimated by parentage analysis. Demographic and ecological monitoring was conducted through inventories in sampling plots.
- Reproductive success of parental trees was higher in *Q. petraea* than in *Q. robur*, and sapling densities were also larger in *Q. petraea*, leading to an expansion of *Q. petraea* (50% to 67% of the area). Admixed trees resulting from introgression between both species were more frequent under the *Q. robur* canopy.
- Competitive exclusion and genetic introgression are the underlying mechanisms favoring the expansion of *Q. petraea*. We anticipate that in mixed *Q. petraea/Q. robur* stands, under current ongoing environmental change, these processes will be enhanced.

Keywords:

Competitive exclusion, introgression, *Quercus robur*, *Q. petraea*, recruitment, succession.

Introduction

Tree species compositions are altered in forest ecosystems as a result of species interactions, natural disturbances and human interferences (Frehlich, 2016). The issue of species changes has received recently increased attention in the context of climate change, but human interferences cannot be dismissed as they can hardly be disentangled from climatic disturbances if both produced confounding effects. There are numerous reviews suggesting that modifications of temperature and precipitations will reshape tree distributions (Ruis-Labourdette *et al.*, 2012; Pucko *et al.*, 2012; Ozolincˇius *et al.,* 2014), or change forest succession (Laflower *et al.*, 2015). These concerns have triggered research in modeling approaches aiming at predicting species distributions under contrasting climatic scenarios (Iverson 2008; Cheaib *et al*., 2012; Wang *et al*., 2016). In contrast to the comprehensive research going on in modeling approaches, little evidence is available from field observation of tree species composition. Indeed, *in situ* assessments of recent shifts of tree distributions are very limited under lowland conditions (Walther *et al.*, 2005; Delzon *et al.*, 2012) while a few more focused on elevational gradients (Kullman, 2002; Peñuelas & Boada, 2003; Lenoir *et al.*, 2008; Bodin *et al*., 2013). Such observations are usually based on the comparison of vegetation inventories over several decades, which do not allow to disentangle the various causes of differential migration of the species (but see Monleon & Lintz, 2015). The inherent ecological processes driving changes in species composition can only be dissected if observations are conducted at the spatiotemporal scale where they are acting. If climate change is indeed accelerating species shifts, their occurrence should be visible at the stand level, and should have been more pronounced during the last decades, when mean temperatures were raised at unusual levels. Following this reasoning, we monitored species composition in two successive generations at the single tree level in a mixed stand of *Quercus petraea* and *Quercus robur,* and targeted in our analysis the changes in species occupancy from one generation to the next. The expected response in distribution and growth of the two species as a result of climate change has been highly debated in recent years (Bobiec *et al.*, 2011; Hlásny *et al.*, 2014). Tree ring analysis indicated that there was a long term increase of radial growth in both species over the last century, but that *Q. petraea* maintained higher growth than *Q. robur* particularly during dry years (Becker *et al.,* 1994; Friedrichs *et al.*, 2009). Together with earlier observations regarding the ecological requirements of the two species (Levy *et al.*, 1992), these authors predicted a steady retreat of *Q. robur* and an expansion of *Q. petraea* if current climatic trends will continue. Indeed, it has been repeatedly observed in mixed oak stands that the former species was more prone to decline than the latter

following the severe summer droughts that occurred from the mid-seventies onward (Durand *et al.*, 1983; Lévy *et al.*, 1994). These field investigations aiming at comparing the two species for their response to climate were mainly based on combined visual inspection of crown status and retrospective analysis of tree ring width of adult trees. However, to date no assessments have been made on the recruitment success of the two species during the critical phase of natural regeneration. The difficulty to reliably identify the two species at young stages has probably precluded such studies. Finally the two species have different successional status which may also alter their spatial distribution in mixed stands. It is well known that *Q. robur* is more a pioneer type species colonizing open areas, while *Q. petraea* is rather a late successional species establishing in areas already occupied by *Q. robur*. It was also shown that hybridization between the two species reinforces the succession dynamics (Lepais & Gerber, 2011). Earlier observations of reproduction under natural conditions suggested indeed that interspecific pollination is asymmetric with pollen of *Q. petraea* more frequently pollinating *Q. robur* than vice versa (Chybicki & Burczyck, 2013; Lagache *et al.*, 2014). These differences in interspecific sexual barriers facilitate the progressive invasion of *Q. petraea*, the late successional species into *Q. robur* the more pioneer species (Petit *et al.*, 2003). While asymmetric reproduction has now been observed *in natura* by parentage analysis, no demographic and genetic survey in mixed stands has confirmed the progressive invasion due to introgression. Clearly, one should expect a preferential distribution of hybrids and introgressed seedlings under the *Q. robur* canopy rather than under the *Q. petraea* canopy. To sum up, there are pending issues regarding the regeneration dynamics and species spatial distribution in mixed *Q. petraea*/*Q. robur* stands. On the one hand, it is unclear whether the predicted higher sensitivity of *Q. robur* to drought has constrained its recruitment during recent years, when summer drought was more frequent. On the other hand, the succession of *Q. robur* by *Q. petraea* reinforced by introgression has yet to be observed under natural conditions. In this study, we addressed explicitly these pending issues. We assessed the species reproductive success and recruitment over two successive generations in a mixed *Q. petraea*/*Q. robur* stand at the individual tree level using genetic fingerprints. This approach allowed us to compare the spatial distribution of both species over two successive generations at a very fine scale, thus allowing to retrace shifts of fine scale distribution. Using genetic fingerprints permitted also to monitor the peculiar dynamics of the admixed seedlings during the regeneration phase and to conclude on the contribution of introgression to the shift of the species distribution.

Material and methods

Study stand and sampling

The study stand is part of the Petite Charnie State Forest located in western France (latitude: 48.085913°N; longitude: 0.168132°W). The State Forest extends over 712 ha and is mainly composed of broadleaves (*Q. petraea*, *Q. robur*, *Fagus sylvatica*), with a slight predominance of *Q. petraea*. The study stand (Fig. S1) is situated in the centre of the Petite Charnie State Forest and covers 5.19 ha (square of 230x226m) and comprised at the beginning of our investigation *Q. petraea* and *Q. robur* in even proportions (Bacilieri *et al.*, 1995). The study plot follows a slight slope oriented from southwest to northeast with a difference of 14 meters in elevation (Methods S1). The two species were distributed along this elevational gradient (Fig. 2), which corresponds to a typical distribution when the two species cohabit (Eaton *et al.,* 2016). Intensive investigations were conducted during the past two decades in this even aged stand addressing spatial genetic structure (Streiff *et al.*, 1998), mating system (Bacilieri *et al.*, 1996), gene flow (Gerber *et al.*, 2014) and hybridization (Lagache *et al.*, 2013). The present study entails now the next generation that resulted from natural regeneration. Natural seeding was carried out using standard silvicultural methods in even aged high oak forests (Jarret, 2004). These methods consist in the opening of the stand by a regeneration felling (or a seed cut) followed by successive additional removal cuts aiming at enhancing seed crop and seedling establishment. A final cut is practiced when the seedling coverage is complete and evenly distributed. In 1989, when the mature trees were 90 years old, a regeneration felling was implemented leaving on the area 426 seed trees (68 trees/ha). The opening of the stand facilitated seedling establishment and was followed by an additional removal cut in 1992 and 1993 leaving 298 standing trees (48 trees/ha). The final clear cut of the 298 trees was done over three years (1998, 2000 and 2001). The final cut was carried out over three years to ease the harvest and manipulation of log samples for later wood and anatomical assessments. Before the final cut, between 1995 and 2001, scions were collected on the 298 remaining trees and grafted in a conservation collection located in a State Nursery of Guéméné Penfao (latitude: 47.631287°N; longitude: 1.892202°W). Natural regeneration resulted in a very dense distribution of seedlings. Given the thinning schedule, seedlings of the natural regeneration resulted of all the successful mating events that occurred between 1989 and 2001. Preexisting seedlings were highly unlikely given the density of the stand before 1989 and the short lifespan of oak seedlings under closed canopy. No silvicultural operation was subsequently conducted between 2001 and 2013; thus the stand composition in 2013 resulted

mainly from natural selection and competition for light and resources. In 2013 a mechanical clearing operation was conducted that removed all trees along linear strips evenly spaced every 9 meters. The width of each strip was 3 meters. In summer 2014, a systematic sampling of 2510 seedlings was made in the regeneration, corresponding to the selection of 1 seedling every 3 to 6 meters along the linear strips. The present study is based on data collected in three cohorts (Table 1):

- The 426 adult mature trees in 1989. This is an exhaustive sampling of all seed trees remaining after the regeneration cut (cohort 1). During the summer 1989, 5 leaves were collected in the upper crown of the trees and 14 morphological traits were assessed (Kremer *et al.*, 2002). This data set was analysed in an earlier study aiming at species assignment based on leaf morphology (Kremer *et al.*, 2002) and is used here for species assignment and mapping of the mature stand.
- The 298 adult mature trees in 1994. This is also an exhaustive sampling of the trees remaining after the two removal cuts in 1992 and 1993 (cohort 2)
- The 2510 seedlings, systematically sampled in the regeneration in 2014 (cohort 3)

For cohort 2 and 3, leaf or bud tissues were collected for DNA extraction and SNP genotyping. For cohort 2, sampled material came from earlier collections done on the standing trees (Mariette ***et al.***, 2002) or more recent collections done on the grafts in 2012 (Lagache ***et al.***, 2013) or in 2015 by ourselves, in the conservation collection. Failed grafting resulted in the loss of 38 trees. Thus cohort 2 ultimately comprised 260 trees in this study. Collection of tissues of cohort 3 was done *in situ* in summer 2015. All trees of all cohorts were mapped by recording their GPS coordinates, using post processed differential corrections. Finally it is worth mentioning that repeated drought events occurred during the last decades (1989-2010) as the saplings of the regeneration established, which resulted in documented decline, growth losses and tree mortalities in western European Forests (Bréda & Badeau, 2008; Carnicer *et al*., 2011). Locally we recorded an increase of 20% of the annual water deficit during the period 1989-2010, in comparison to the period 1955-1988 (data not shown).

Botanical survey, fine scale ecological mapping and demographic inventory

In July 1992, a floristic survey was conducted within 34 plots systematically distributed throughout the study stand. The sampling included 8 survey plots located along the main diagonal from southwest to the northeast which was orthogonal to the slight slope in the

study stand (Bacilieri *et al.*, 1995; Methods S1). Each survey plot consisted of a circular area of 64 m^2. The presence of all vascular plants (pteridophytes and spermatophytes) was recorded within each plot. Altogether 120 species were identified over the whole study area.
The botanical survey data were used to infer key soil characteristics. Bioindication of soil variables were drawn from large databases of species indicator values established for temperate western European forests (Gégout *et al.*, 2003, 2005; Ellenberg *et al.*, 1992, Methods S1). We calculated mean indicator values for the following soil attributes: pH, soil moisture, ratio of carbon to nitrogen (C/N) and organic matter content for each sampling plot. On average, 26, 22, 22 and 17 species per plot were available for the calculation of mean indicator values for pH, soil moisture, C/N and organic matter content, respectively.
These variables were then further downscaled to a single tree level after kriging (Methods S1).

In July 2016, a demographic survey was conducted to assess sapling densities in cohort 3 to derive census estimates (Methods S1). The survey was based on a systematic sampling of 49 square survey plots distributed according to a grid system throughout the study stand. The area of each plot was 25 m^2 on average and all saplings present in a given plot were counted.

DNA extraction and SNP genotyping

DNA of parental trees of cohort 2 was previously extracted by Lagache *et al.* (2013) and Mariette *et al.* (2002). DNA of seedlings of cohort 3 was isolated from 5 punches of leaves using the Invisorb DNA plant HTS 96 kit (Invitek GmbH, Berlin, Germany), according to manufacturer's recommendations.

Four medium-throughput SNP genotyping assays were developed using a MassARRAY® System (Agena Bioscience™) and iPLEX® chemistry. Three multiplexed assays were designed (W1 and W2 with 40 SNPs and W3 with 29 SNPs) from a collection of oak SNPs previously validated in Lepoittevin *et al.* (2015) and selected according to their MAF (Minor Allele Frequency) > 30 % and their evenly distribution along the twelve *Quercus* linkage groups (Bodénès *et al.*, 2016). Another multiplex of 17 SNPs (W4) selected for their interspecific differentiation between *Q. petraea* and *Q. robur* in Guichoux *et al.* (2013) was added to the SNP panel. Overall, a total of 126 SNPs distributed in four multiplexes were finally used to genotype all individuals (260 parents of cohort 2 and 2490 offspring of cohort

3). Twenty samples of cohort 3 had to be discarded before extraction due to the poor quality of the leaf material collected.

The iPLEX reactions use a first PCR to amplify specific regions containing a SNP. A second PCR amplification, named extension reaction, is performed using a mass-labeled nucleotide which is added in the SNP position. Primer extension products are placed on a silicon chip, with each sample affixed to a spot containing the multiplex for all SNPs. The chip is then run in a mass spectrometer where the primer mass plus the SNP nucleotide mass is determined. In the assay, nucleotide base calls for SNPs were exported and assessed in MassARRAY® TYPER 4.0 genotyping software. Base calls were automatically determined and then all plots were manually verified. Each SNP locus was recorded as successful after visual inspection of the scatter plots (Methods S2).

Species assignment

We assigned trees of cohorts 2 and 3 to their relative species (*Q. robur* or *Q. petraea*) using version 2.3.3 of STRUCTURE (Pritchard *et al*., 2000). The analysis was conducted over the whole data set comprising trees of cohort 2 and 3. The number of groups tested was K = 1 to 8. The admixture model with correlated allele frequencies was used. A burn-in of 250 000 steps was followed by a Markov chain Monte Carlo repetition of 500 000 steps, with 30 iterations. The most likely numbers of populations (K) was estimated using the Ln probability of the data according to Pritchard *et al. (*2000) and the Delta-K method by Evanno *et al.* (2005) as implemented in STRUCTURE HARVESTER (Earl & von Holdt, 2012). The most probable number of populations was 2 according to the Delta-K value and the mean Ln probability of the data. Runs generated at K=2 were clustered and averaged using CLUMPAK (Kopelman *et al.*, 2015).

Individual trees were assigned to the two species according to the value of the admixture coefficient (q). Assignment was made in three groups according to different threshold values of q: *Q. petraea* purebreds (q >=0.9), admixed trees (q varying between 0.1–0.9) and *Q. robur* purebreds (q <= 0.1). The choice of the threshold for q was based on the results of a simulation study specifically designed for species assignment in interspecific oak mixtures (Neophytou, 2014). This study showed that the performance of assignment (efficiency and accuracy) was highest with STRUCTURE when the threshold of q was set to 0.90. The study indeed indicated that 99% of purebreds were correctly assigned to their taxonomic groups

(either *Q.petraea* or *Q.robur*), and 85 % of the admixed were also correctly assigned (Neophytou, 2014). Although the simulations were based on allele frequencies of different markers than ours, but with similar levels of interspecific species differentiation to ours, we assume that the chosen threshold level (q=0.9 and q=0.1) would provide similar levels of performance in our study. Considering the admixed individuals, q values varying between 0.375 and 0.625 would be expected for F1 hybrids and values lower than 0.375 or larger than 0.625 for backcrossings. However the simulation study also indicated that q values of first generation hybrids and backcrossed individuals largely overlapped (Neophytou, 2014). Therefore in what follows, these individuals (hybrids+backcrossed) will be called "admixed" individuals.

A retrospective assignment to the two species was also conducted on cohort 1 using leaf morphological traits, after comparing the leaf morphological variation of trees of cohort 2 with their admixture coefficient (Methods S3). However leaf morphological data did not allow to assign trees to the admixed group (Methods S3).

Parentage analysis and reproductive success

Parentage analysis was conducted using CERVUS 3.0.7 (Marshall *et al.*, 1998) between adult trees of cohort 2 and offspring saplings in cohort 3. For each offspring tested, parentage is assigned to the most-likely candidate parent with a pre-determined level of confidence.

CERVUS uses simulations to evaluate the confidence in assignment of parentage to the most likely candidate parent. As well as using observed allele frequencies the simulation takes into account the number of candidate parents (1 000), the proportion of candidate parents sampled (30%), completeness of genetic typing (94%) and estimated frequency of typing error (0.01) when generating genotypes. The number of simulated offspring was set at 10000 and the minimum number of loci typed by individual was set at 40.

The program allows genotyping errors and assigns parents despite mismatching loci when confidence is otherwise high. It can be argued that only the most confident data should be used in parental analysis, and thus only stringent criteria allowing no mismatches should be used to avoid false assignment. Thereby, stringent parameter analyses were conducted assuming no errors in the genotypes (a strict exclusion analysis: 0.0 error rate) and a high confidence level (95%). Results of the parentage analysis were then used to calculate the reproductive success of each parent tree, as a male or a female parent. The method does not allow in our case to infer the sex of the parent tree in the different matings. As parentage

analysis was done on a systematic sampling of saplings, the number of saplings assigned to a given tree does not correspond to the absolute reproductive success of that tree (i.e. the total number of saplings the tree produced). In what follows it will therefore be called relative reproductive success.

Data resource

Leaf morphology data and SNP genotypic data for all samples collected from the Petite Charnie forest will be were stored in DRYAD. The leaf morphology file contains 15 traits described in the “read me” tab. The genotype file contains for 82 SNP loci the genotypes for 2490 offspring and 260 parents.

Results

SNP genotyping and SNP diversity.

Four multiplexes containing 126 SNP were used for genotyping 260 and 2490 parental and offspring trees respectively. After analyzing each SNP profile individually (Methods S2), we assessed as successful 82 SNP loci (23 for W1, 25 for W2, 18 for W3 and 16 for W4). On average, 80 loci were genotyped for parental trees (Min: 45 / Max: 82) and 77 loci for offspring (Min: 32 / Max: 82). The call rate (ratio of number of assigned genotypes to the total number of genotypes) for parental and offspring samples was 97% and 94% respectively. Forty-four SNP have been entirely discarded after visual inspection of the scatter plots because of failed clustering (low intensity magnitude, more than 3 clusters, too weak or no amplification, Methods S2). As expected, given the selection criteria of the SNP, SNP alleles were rather evenly distributed, with mean MAF values of 0.4 in the parental population (cohort 2) and the offspring population (cohort 3) (Table S1). No SNP locus was found monomorphic. There was no significant differentiation between SNP frequencies between cohort 2 and 3 as the overall Fst between both cohorts amounted to 0.008.

Species assignment

We used the Bayesian clustering analysis STRUCTURE with the admixture model to assign individuals trees to their species according to the admixture coefficient of each tree (see methods). In each cohort, the distribution of q values indicated that individuals clustered mostly in the two purebred groups (Table 2, Fig. S2).

According to STRUCTURE, we identified in cohort 2, 135 *Quercus robur* (52%), 110 *Quercus petraea* (42%) and 15 admixed individuals (6%). In cohort 3, the 2490 saplings were subdivided into 820 *Q. robur* (33%), 1570 *Q. petraea* (63%) and 100 admixed (4%) offspring. (Table 2, Fig. S2). Admixed trees were equally distributed below and above the q=0.5 value in cohort 2.

Species assignments based on morphological traits (morpho groups) and assisted by genetic SNP fingerprints was also done in cohort 1 (Methods S3). By using a threshold value of the first principal component (PCA1) of 1, 196 (46%) trees were assigned to *Q. petraea* and 226 (54%) to *Q. robur* (Table 2). As admixed individuals could not be assigned using morphological traits, the two morpho groups of cohort 1 also comprised admixed individuals. Assuming that admixed individuals were not preferentially selected during the removal cut in 1992-1993 and equally distributed between the two morpho groups, the corrected numbers of

Q. petraea and *Q. robur* in cohort 1 are 184 (44%) and 212 (50%). After species assignment in cohort 2 and 3 we confirmed that SNPs of multiplex W4, that were previously selected for their interspecific differentiation between *Q. petraea* and *Q. robur* (Guichoux *et al.*, 2013) did indeed exhibit higher interspecific Fst values (Table S1)

Spatial distribution of the species

We used the threshold value of PCA1=1 in cohort1 and the threshold value of q=0.5 in cohort 2 and cohort 3 to map the species distribution in the study stand by plotting contour lines. The plotting of the contour line separating the two species ignores the admixed individuals. The plotting is done for comparative purposes within the three cohorts separately and thus could only be based on the pure species level, as admixed individuals could not be assigned in cohort 1. Mapping of the contour lines was done by plotting kriging interpolated values of PCA1=1 and q=0.5 (Fig. 1a, b, c). Using this procedure, areas occupied by the two species could be estimated. Overall there was a clear shift from cohort 1 to cohort 3 resulting in the increase of the area occupied by *Quercus petraea* from 50% to 67 % of the whole area (Table 3).

Plotting contour lines between the two species zones should not ignore the existence of isolated trees of one species that are present in the zone of the other species, which we will call "outlier" trees, and that may have an important contribution to the succession and recruitment dynamics. It is worthwhile noticing that the density of "outlier" *Q. petraea* within the *Q. robur* zone is lower than the reciprocal in both adult cohorts 1 and 2 (11.5/ha *vs* 14.3/ha in cohort 1 and 4.4/ha *vs* 7.9/ha in cohort 2). These results further highlight the higher recruitment success in cohort 3 of *Q. petraea,* as the presence of outlier *Q. robur* parental trees did not constrain the expansion of *Q. petraea*. On the contrary outlier *Q. petraea* trees, despite less frequent, were clearly instrumental to the expansion of *Q. petraea* as can be seen by comparing Fig. 1a and 1b (arrows on the Fig. 1a). Indeed, substantial expansion of *Q. petraea* from cohort 1 to cohort 3 occurred around the *Q. petraea* "outlier" trees of cohort 1.

Finally, although admixed trees were not taken into account for plotting the contour line of the two species, we positioned the admixed individuals within the *Q. petraea* and *Q. robur* zones for cohort 2 (Fig. 2a) and cohort 3 (Fig. 2b). Although the number of admixed adult trees in cohort 2 is low, there is a consistent trend towards higher densities of admixed trees in the *Q. robur* zone than in the *Q. petraea* zone. Expressed in tree densities there are 2.54 times more admixed trees in the *Q. robur* zone than in the *Q.petraea* zone in cohort 2, and 2.78

353 times more in cohort 3 (Fig. 2). When present in the the *Q. petraea* zone, admixed trees of
354 cohort 3 were preferentially located under the canopy *Q. robur* outlier trees (comparison of
355 Fig. 2b and 1a).
356
357 Demographic and reproductive monitoring
358
359 We used the data of the demographic survey to estimate sapling densities in cohort 3 for each
360 species. As species assignment was not feasible for all saplings, we assumed that all saplings
361 within a survey plot belonged to the same species. We derived average estimates of densities
362 by bulking data over all survey plots present in the *Q. petraea* zone, and those present in the
363 *Q.robur* zone. Mean sapling density of *Q. petraea* was slightly higher (8758 saplings/ha for
364 *Q. petreaa vs* 6822/ha for *Q. robur,* Fig. 3a) and median values ranged between 8071/ha and
365 3470/ha for the two species (Wilcoxon rank sum test, p=0.37). These differences were not
366 significant because of the extreme variation among inventory plots in both species (roughly
367 from 300 to 30 000 in *Q.petraea* vs 300 to 22 000 in *Q. robur*), with no significant differences
368 in the variance of sampling densities between both species. Pooling the data of sapling
369 densities and distribution areas of the two species (Table 3) resulted in a total census number
370 of 30390 (72%) saplings of *Q. petraea* and 11802 (28%) of *Q. robur* in cohort 3.

371 Similar results were also obtained for relative reproductive success of parental trees in cohort
372 2 estimated by parentage analysis based on the systematic sampling of 2490 saplings in cohort
373 3. Parentage analysis was conducted for 2487 saplings as 3 were discarded because less than
374 40 SNPs could be reliably genotyped (Table 4). A total of 1453 saplings were assigned to at
375 least one parent (58%). Only one parent was identified for 45% of the offspring (n= 1126),
376 while two parents were found for 13% of offspring (n= 327). Only 17 adult trees (6.5%)
377 among the 260 did not produce any offspring among the 2487 saplings investigated.

378 Relative reproductive success of *Q. robur* was on average lower than in *Q. petraea* (6.2
379 versus 8.1 offspring per parent (Wilcoxon rank sum test, p=0.09) and its distribution was
380 skewed in both species towards larger values (Fig. 3b). The maximum number of offspring
381 per parent varied from 0 to 26 for *Q. robur* and from 0 to 53 for *Q. petraea*. Admixed parent
382 trees exhibit less reproductive success then the pure species trees (3.5 offspring on average
383 per parent, varying from 0 to 14). Relative reproductive success seems to be slightly higher in
384 the north central part of the study stand. Higher reproductive successes are also visible in *Q.*

petraea along the contour line with *Q. robur* particularly near zones where *Q. petraea* expanded (Fig. 4 and 1b).

Ecological preferences of the species and admixed individuals.

Floristic indicators of pH, soil moisture, C/N ratio and organic matter showed significant differences between the two species, while the admixed group was usually intermediate between both species. We illustrate these results for cohort 3 where the sample sizes were the largest (Fig. 5) but similar results were also obtained in cohort 1 and 2. Mean topographic elevation of the two species and the admixed group were also different. A striking feature of the distribution of the ecological indicators is the larger density distribution of *Q. robur* in comparison to *Q. petraea* regardless of the indicators. A further remarkable observation is that the distribution of the admixed trees overlapped generally with the distribution of *Q.robur* (Fig. 5). These data corroborate the just reported spatial observations that admixed individual occur more frequently under *Q.robur* canopy than under *Q. petraea* canopy. Finally we compared also within each species changes of ecological indicators across all 3 cohorts. Differences were statistically significant for all ecological indicators for *Q. robur*, towards decreasing elevation and C/N, higher pH values, soil moisture and organic matter. The converse was observed in *Q. petraea*, but differences were only significant for the level of organic matter (data not shown).

Discussion

This survey provides unprecedented results on the regeneration dynamics and on the changes of species occupancy in one single generation in a mixed oak stand *(Q.petraea/Q.robur)*. It is important to recall that the regeneration phase cumulated 12 years of reproduction (1989-2001) followed by thirteen years of natural selection and competition (2001-2014) within the seedling/sapling cohort of the two species. Thus the results obtained are free from year to year variation that can impact the flowering, pollination, or seed crop. There is no background information on the frequency of flowering or pollination, but reasonable good acorn crops from a silvicultural point of view occur every three years in this part of France (Jarret, 2004). By combining ecological, genetic and demographic monitoring over two generations we were able to depict two processes that drive the species distribution under mixed configuration: competitive exclusion and introgression. Both are acting at different tempos, the former may shape the expansion/retraction of a species within one generation, whereas the latter may take

420 a few generations. We showed in this study that both processes were acting in the same
421 direction towards the expansion of *Q. petraea* at the expense of *Q. robur*.
422
423 Replacement of *Q. robur* by *Q. petraea* due to competitive exclusion
424
425 Our results clearly showed that *Q. petraea* has expanded at the local scale, both numerically
426 and spatially (Table 2; Fig. 1). Differences in recruitment success might be caused by species
427 differences in seed crop, in dispersal, and in response to interspecific competition. Given the
428 present sapling densities, we suspect slightly higher seed crops in *Q. petraea* than in *Q. robur*
429 but differential survival may also be responsible for the present demographic differences.
430 Concerning dispersal, earlier investigations based on parentage analysis conducted in the
431 same stand and in others showed very limited and highly variable dispersal distances of
432 acorns (on average less than 100 meters) (Gerber *et al.*, 2014). In two other examples, 90% of
433 the acorns were dispersed less than 10 to 40 meters from the seed tree (Chybicki & Burczyck,
434 2013). But there was no strong evidence for species variation in seed dispersal. Competition
435 experiments show however contrasting differences between the two species. Généré and Le
436 Bouler (1996) and Guibert and Généré (2000) conducted a long lasting experiment where
437 acorns of the two species were sown in controlled mixtures in nursery beds under high
438 densities. After one year in the nursery, the seedlings were transferred in the forest in densities
439 mimicking natural regeneration while maintaining the same mixtures between species than in
440 the nursery. The whole experiment was replicated in three different forests and height growth
441 and survival was assessed over four successive years in the field. During the nursery step, the
442 density of seedlings of *Q. robur* increased after one year in all mixture modalities. In the field
443 experiments, regardless of the year and the forest, mortality was systematically higher in *Q.*
444 *petraea* than in *Q. robur*, in pure and in mixed conditions. Cumulative height growth of the
445 surviving seedlings was also higher in *Q. robur* than in *Q. petraea*. But more interestingly, the
446 height growth performance did not change in *Q. petraea* between pure and mixed conditions,
447 while growth systematically increased in *Q. robur* under mixed conditions in all three forests.
448 Superior juvenile growth of *Q. robur* was also observed in seedling by seedling mixtures with
449 *Q. petraea* (Landergott *et al.*, 2012). Clearly the consistent outcome of these experiments was
450 a demographic increase and an increase of growth of *Q.robur* in mixed plantations, thus
451 demonstrating the higher competitive ability of this species at least at a juvenile stage
452 (Guibert & Généré, 2000). This is consistent with the pioneering status of *Q. robur*
453 characterized by its strong ability for rapid seedling establishment.

How can these observations be reconciled with our own data that clearly indicated an opposite trend? On the one hand, one may advocate that the superior growth and competitive ability of *Q.robur* is transient and more pronounced at the very juvenile stage (Ponton *et al.*, 2002). Support for this hypothesis comes from the comparative tree ring analysis in older stands, which consistently show larger ring width in *Q. petraea* than *Q. robur* (Becker *et al.*, 1994; Friedrichs *et al.*, 2009; Levy *et al.*, 1992) and from observation of adult survival (Ponton *et al.*, 2002). These time trends in growth suggest a tipping point where the growth curves of both species cross each other, and when *Q. petraea* becomes a stronger competitor than *Q. robur*. Our results suggest that this tipping point occur sometime between the very juvenile phase (before age 5) and age 15. A second hypothesis is that the growth and competitive ability of *Q. robur* in the juvenile phase can be reduced under drought conditions (Fonti *et al.*, 2013). As stated and shown experimentally by these authors "*Q. robur* is more competitive under favorable growing conditions but at risk under severe exposition to drought". Earlier experiments comparing the two species under different levels of drought confirm indeed that *Q. petraea* sustained stress better than *Q. robur* in terms of tolerance to drought (Vivin *et al.*,1993; Arend *et al.*, 2013) and to higher temperature (Hu *et al.*, 2015). To conclude, we suspect that differential response to the severe droughts that have accumulated during the last decade, and differential competitive ability at different life stage may be responsible for the spatial shift of *Q. petraea* into *Q. robur*. It is unclear however whether these shifts were also reinforced by past human interferences. There is historical documentary evidence that local forests were managed for producing fuel wood for the forge industry during the 18th to 19th century (Pesche, 1829). Woodlands were treated as short term coppice to maintain high level of wood production (Dufour, 1984). While coppice does not change the genetic or species composition, it is more favorable to *Q. robur* than to *Q. petraea*. Thus forest management may have artificially fostered *Q. robur*. As a result, one may also interpret the contemporary expansion of *Q. petraea* as a feedback to its ecological niche, which is facilitated by climate change.

Replacement of *Q. robur* by *Q. petraea* driven by introgression

We found evidence of ongoing succession with *Q. petraea* replacing *Q.robur* driven by the preferential unidirectional introgression between the two species (Petit *et al.*, 2003). Our conclusions are based on the distribution of admixed individuals within the mixed stand that

occur more frequently within the *Q. robur* zone than within the *Q. petraea* zone. We also noticed that admixed saplings present in the *Q. petraea* zone, were preferentially located near "outlier" *Q. robur* parental trees that were surrounded by *Q. petraea* trees and thus prone to be hybridized by *Q. petraea* (Fig. 1a and 1b). Under such circumstances unidirectional hybridization due to mating barriers between the two species is indeed reinforced by the demographic unbalanced species composition of the neighborhood (more *Q. petraea* trees surrounding outlier *Q. robur* trees). Genetic surveys aiming at inventorying admixed individuals in mixed stands using the same molecular technique as ours has confirmed that admixed proportions may provide a relevant clue to the colonization dynamics (Beatty *et al.*, 2016; Neophytou *et al.*, 2015). These two studies showed indeed that admixed proportions were higher at the northern margin of the distribution in Ireland (Beatty *et al*., 2016), as compared to more central areas as Germany (Neophytou *et al.*, 2015). These studies compared extant spatial distant populations undergoing different colonization dynamics. There is one reported diachronic study that aimed to track introgression triggering succession dynamics in a mixed *Q.petraea/Q.robur* stand (Boratyński *et al.*, 2010). Using a similar approach than ours but based on morphological traits, these authors found that the proportion of hybrids was larger under *Q. robur* canopy than under a *Q. petraea* canopy. Furthermore their demographic investigations showed also a higher recruitment success at age 17 of *Q. petraea.* Their results were altogether similar to ours, despite the different fingerprinting technique used. Further insights into the tempo of succession may be obtained by recording more precisely the level of introgression within the admixed saplings, by using a larger number of molecular markers. One would expect a larger range of introgression values within the *Q. robur* zone, as a signature of the successive backcrosses predicted by the succession dynamics. Our study did not allow to distinguish between first generation hybrids and later backcrossed individuals, as our fingerprinting was not resolutive enough. While our observations suggest that succession through introgression is actually going on, they also indicate that this process remains overall limited, as only 4% of admixed saplings were present in cohort 3 (Table 2). It is surprising that this proportion was actually even lower than the one observed in the previous adult generation (6 % in cohort 2). We suspect that some of introgressed individuals were eliminated by natural selection or competition with pure breds during earlier stages. Indeed hybridization rates estimated at the acorn stage were on average much higher (sometimes up to 40%) in different European stands (Gerber *et al*., 2014), but much lower (0.1 to 3%) in one single year monitoring in our study stand (Lagache *et al.*, 2013). Nevertheless the proportion of introgressed individuals is similar to values recorded in

other stands in central Europe and using the same technique (3% in the Rhine Valley, Neophytou *et al.*, 2015; 5.7% in the South of France, Lepais *et al.*, 2009). If those few percent of introgressed trees become ultimately *Q. petraea* once the backcrossings are completed, they will only contribute to a limited part of the *Q. petraea* expansion, in comparison to the recruitment success that was discussed earlier.

As a conclusion, we anticipate that in mixed *Q.petraea/Q.robur* stands, under current ongoing environmental change, *Q. petraea* will potentially replace *Q. robur* particularly under sites that become drier and that were formely prone to *Q.robur*. The main driver of this shift is likely to be the changes in the site conditions that modify species competition and responses rather than succession due to introgression.

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Author contributions

A.K. designed the study. L.T. carried out the field sampling, and demographic survey with contributions of the technical staff. E.C. designed and implemented the genotyping procedure. F.E. contributed to the data flow and management. A.D. installed the study plot at the beginning and carried out the ecological monitoring. L.T did the demographic and ecological analysis with the help of J.L.D. and V.B. E.C. did the species assignment and parentage analysis. A.K., L.T. and E.C. wrote the manuscript, and all other authors reviewed and amended the complete manuscript.

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732 **Figure Titles and legends**

733

734 **Fig.1** Spatial distribution of the trees in the three cohorts and contour line of the two species

735 **Fig. 1a** Cohort 1

736 **Fig. 1b** Cohort 2

737 **Fig. 1c** Cohort 3

738

739 **Fig. 2** Distribution of admixed trees in cohort 2 (Fig. 2a) and in cohort 3 (Fig. 2b)

740

741 **Fig. 3** Box plot of sapling density and relative reproductive success in *Q. petraea* and *Q.*
742 *robur*

743 **Fig. 3a** Sapling density (cohort 3)

744 **Fig. 3b** Relative reproductive success of parental trees in cohort 2

745

746 **Fig. 4** Distribution of the relative reproductive success

747 Centres of circles indicate the position of parental trees in cohort 2, while the size of the circle
748 is proportional to the relative reproductive success of the parental trees (largest circle: 53 and
749 smallest circle: 0). Thick line represents species separation line in cohort 2.

750

751

752 **Fig. 5** Density distribution of ecological indicator values in the two species and admixed group of
753 cohort 3

754 **Fig.5a** pH

755 **Fig. 5b** Soil moisture

756 **Fig. 5c** Carbon/nitrogen ratio

757 **Fig. 5d** Organic matter

758

759

760 **Table 1** History of silvicultural operations, assessments and sampling conducted in the study
761 stand

762

Year	Silviculture operation	Census Number	Sampling size	Assessments and operations for this study
1989	Regeneration felling	426 (cohort1)	426 (422)* trees	Leaf morphology in cohort1
1992	Botanical survey		36 plots	Ecological mapping of the study stand
1992-1993	Removal cut	298 (cohort2)		
1995 to 2001		298		Scion collection for grafting
1995 to 2001		298	298 (260)* trees	Collection of bud and leaf tissue for DNA extraction in cohort2
1998 to 2001	Final clear cut			
2013	Mechanical systematic cleaning			
2014		> 6000/ha (cohort3)	2510 (2490) * saplings	Collection of bud and leaf tissue for DNA extraction
2015			2490 saplings	SNP genotyping of cohort 2 and 3
2016			49 plots	Assessment of sapling densities in cohort 3

763 Numbers between brackets indicate the ultimate sample sizes used for the analysis of
764 the data, after discarding individuals due to technical constraints and assessment
765 difficulties during phenotyping (cohort 1) or genotyping (cohort 2 and 3)

766

Table 2 Number of trees (in percentage) assigned to the different taxonomic groups in the three cohorts

Taxonomic group	Cohort1	Cohort2	Cohort3
Q. petraea	196 (46%)	110 (42%)	1570 (63%)
Q. robur	226 (54%)	135 (52%)	820 (33%)
Admixed	NA	15 (6%)	100 (4%)

NA: number of admixed individuals could not be estimated given the species assignment method base on leaf morphological traits (see text and Methods S3)

Table 3 Absolute (m^2) and relative (%) areas occupied by the two species in the different cohorts

Species	Cohort1	Cohort2	Cohort3
Q. petraea	25 848 (49.8%)	29 028 (55.9%)	34 652 (66.7%)
Q. robur	26 084 (50.2%)	22 904 (44.1%)	17 280 (33.3%)

783 **Table 4** Statistics of parentage analysis 2.

784

785

	Q. robur	*Q. petraea*	**Admixed**	**Total**
Number of parental trees which have at least one offspring	125	107	11	243
Number of offspring assigned to at least one parent	636	758	59	1453
Mean number of offspring per parental tree *	6.2	8.1	3.5	_
Minimum / maximum number of offspring (per parental tree)	0 / 26	0 / 53	0 / 14	_

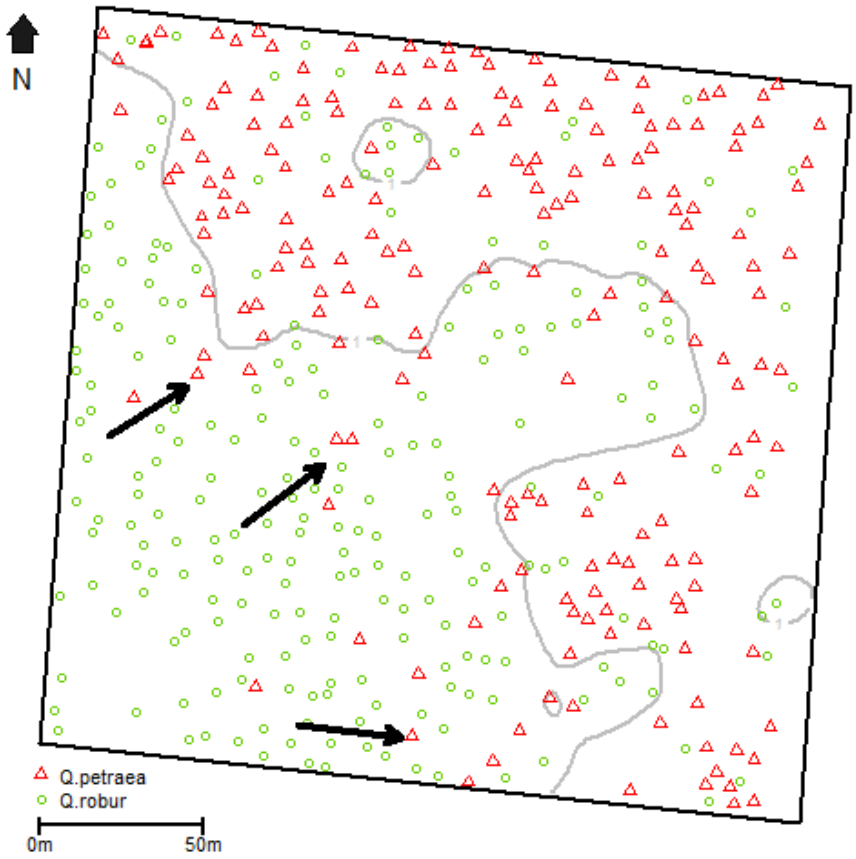
786 *These numbers are not absolute numbers. They are relative to the sapling sampling

787

788

789 **Fig.1** Spatial distribution of the trees in the three cohorts and contour line of the two species

790 **Fig. 1a** Cohort 1



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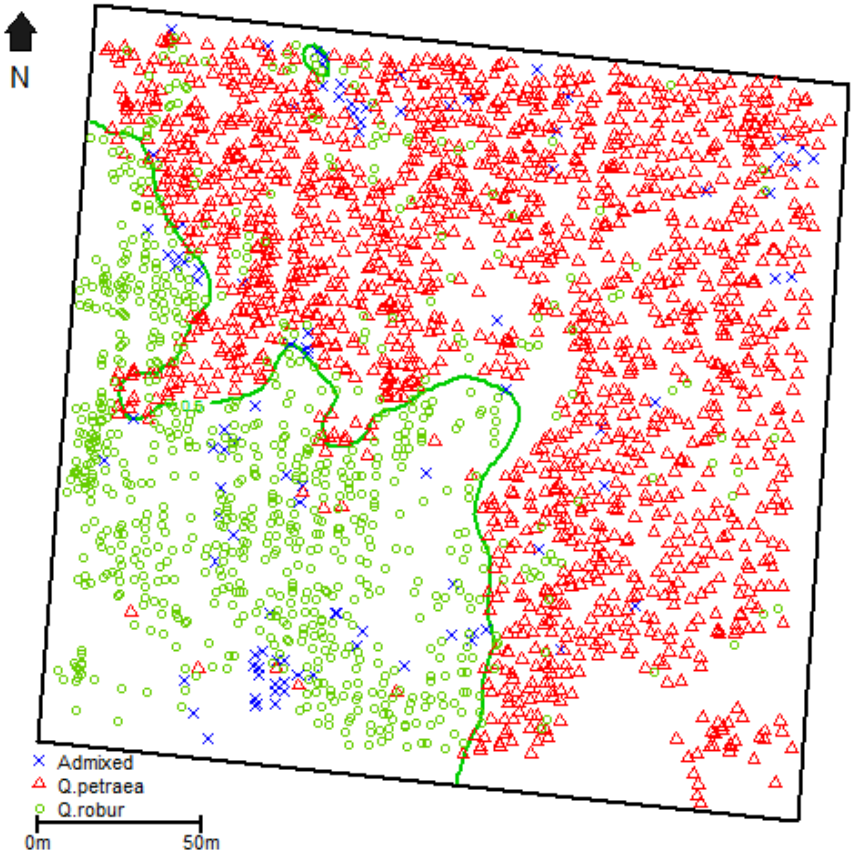
793 **Fig. 1b** Cohort 3



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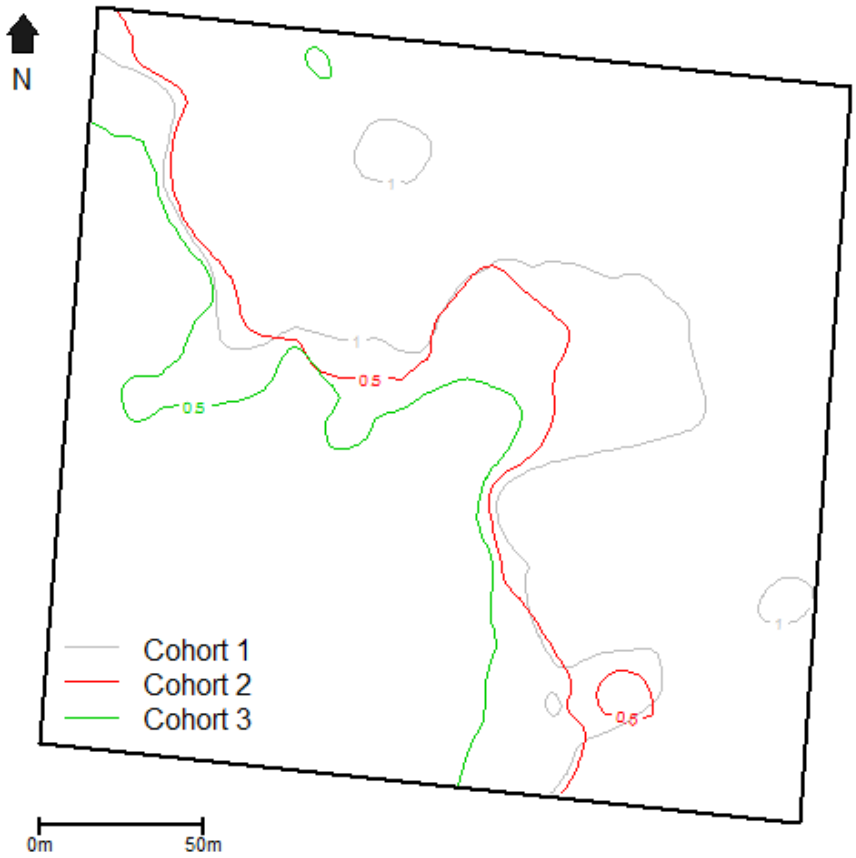
796 **Fig. 1c** Contour lines between the two species in the three cohorts



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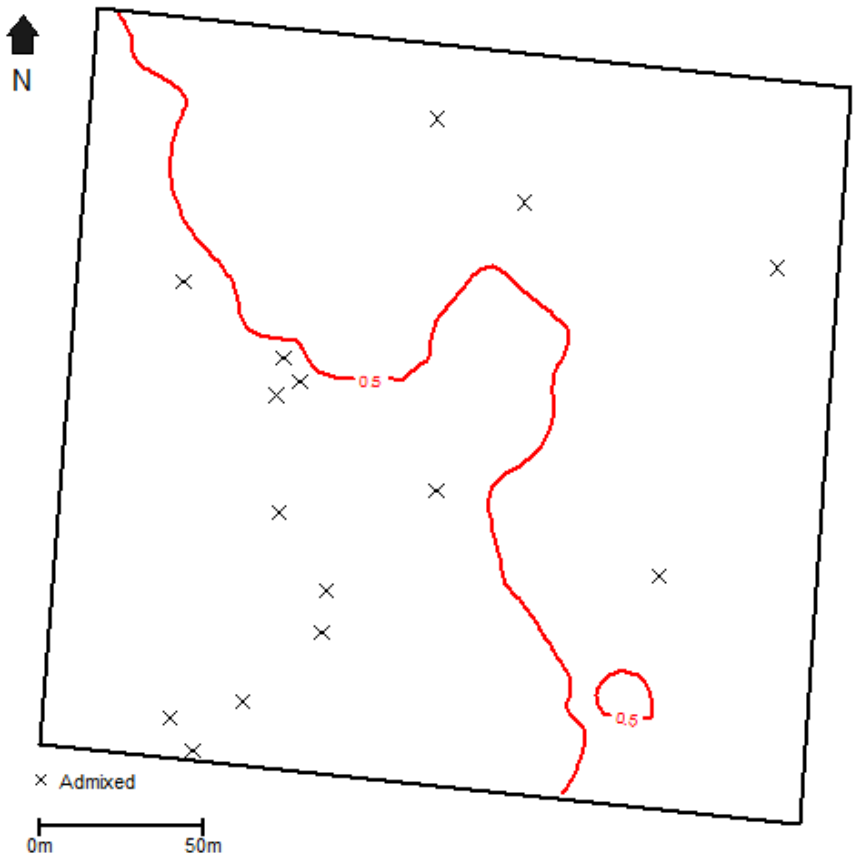
799 **Fig. 2** Distribution of admixed trees in cohort 2 (Fig. 2a) and in cohort 3 (Fig2b)

800

801 **Fig 2a** Distribution of admixed saplings in cohort 2



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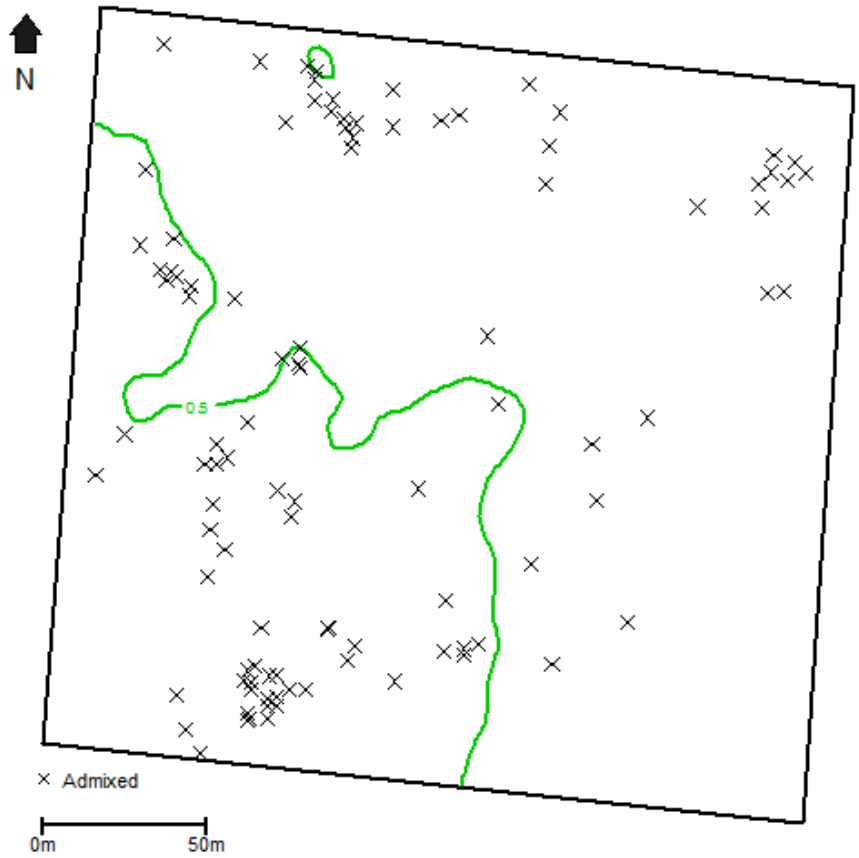
804 **Fig. 2b** Distribution of admixed saplings in cohort 3



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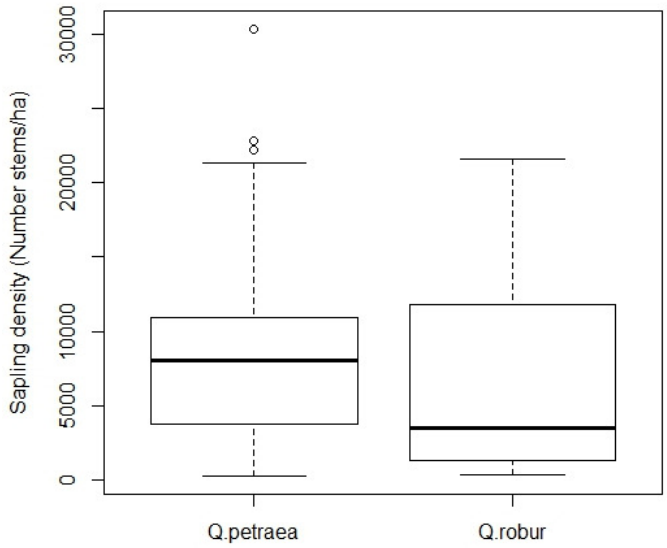
809 **Fig. 3** Box plot of sapling density and relative reproductive success in *Q. petraea* and *Q.*
810 *robur*

811 **Fig. 3a** Sapling density (cohort 3)



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814 **Fig. 3b** Relative reproductive success of parental trees in cohort 2

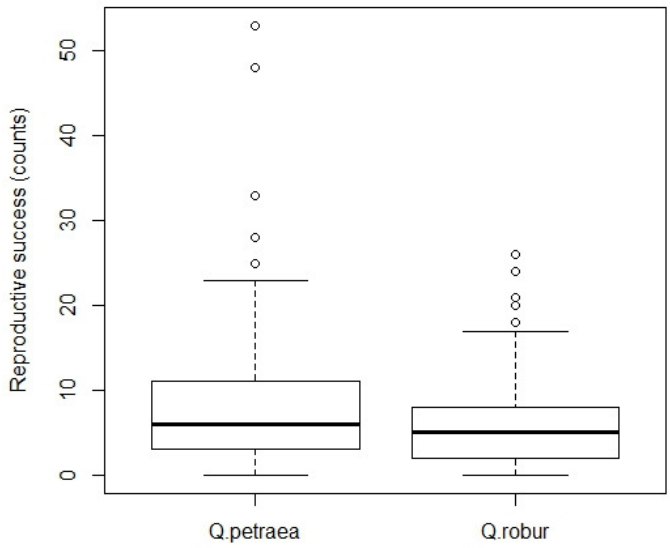

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Fig. 4 Distribution of the relative reproductive success

Centres of circles indicate position of parental trees in cohort 2, while the size of the circle is proportional to the relative reproductive success of the parental trees (largest circle: 53 and smallest circle: 0). Thick line represents species separation line in cohort 2.

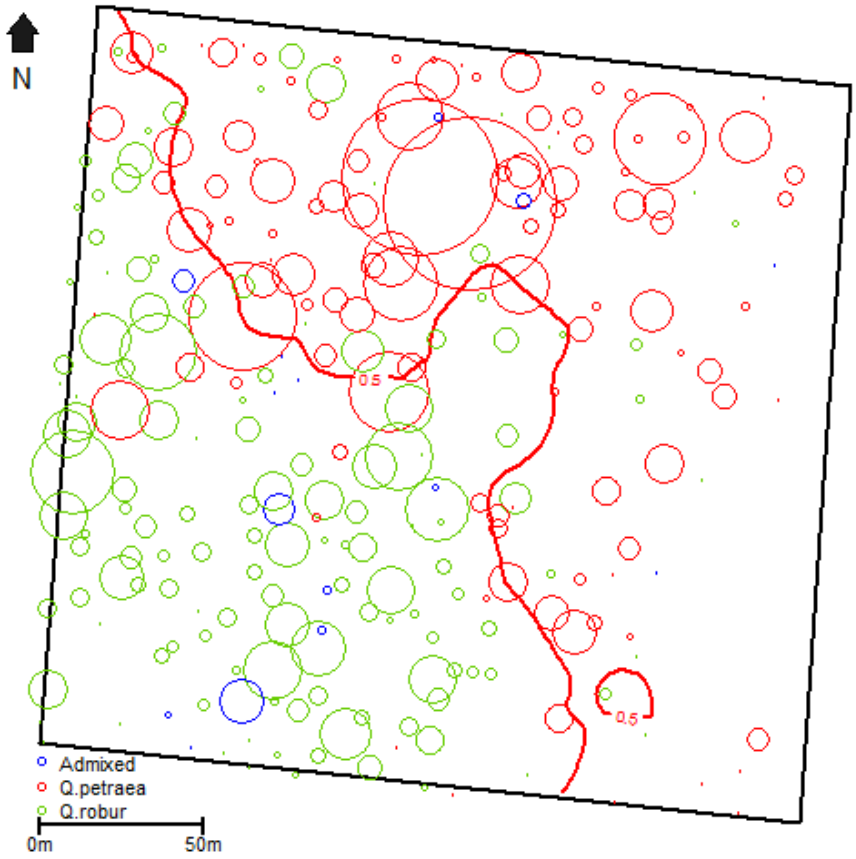
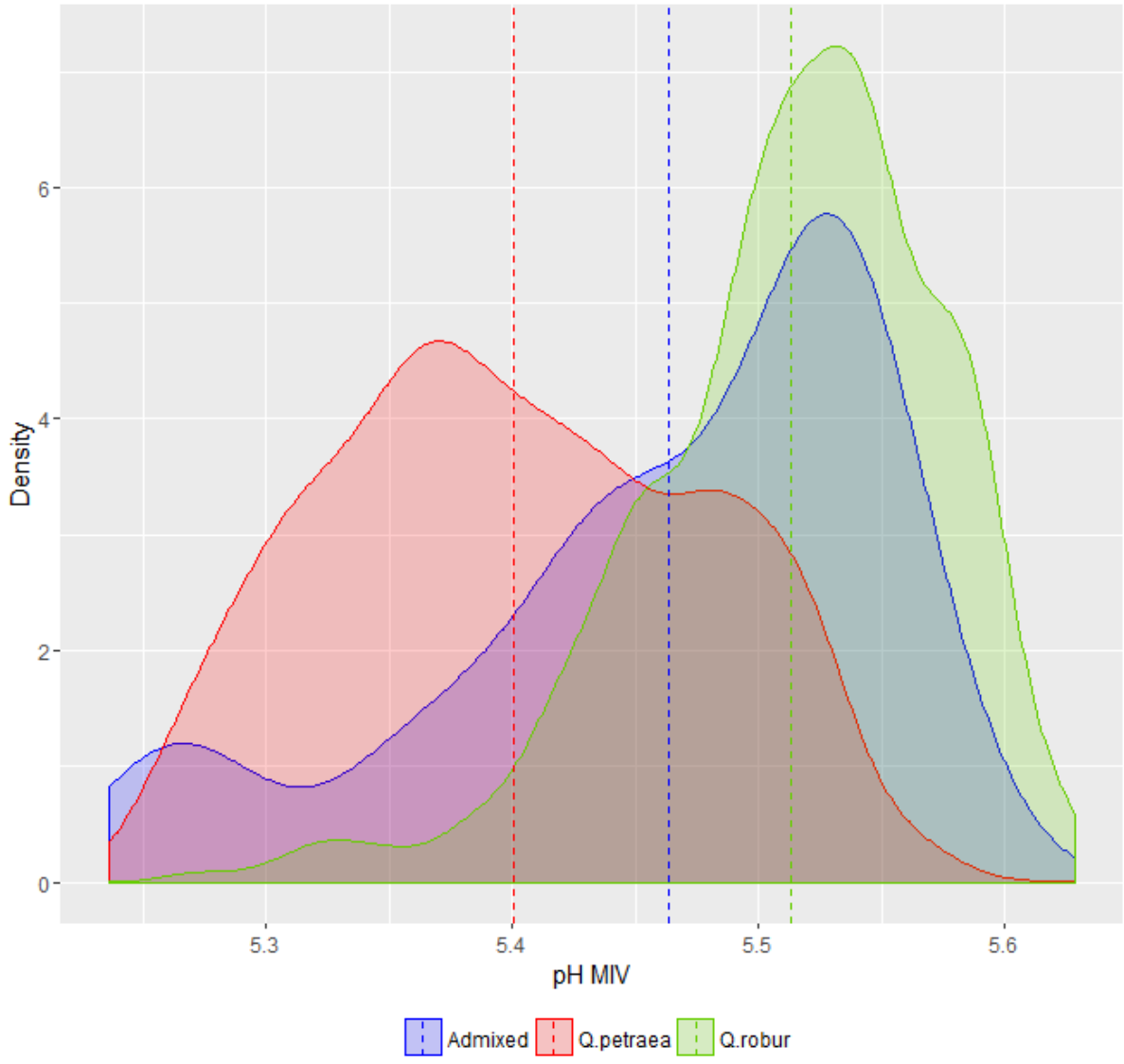
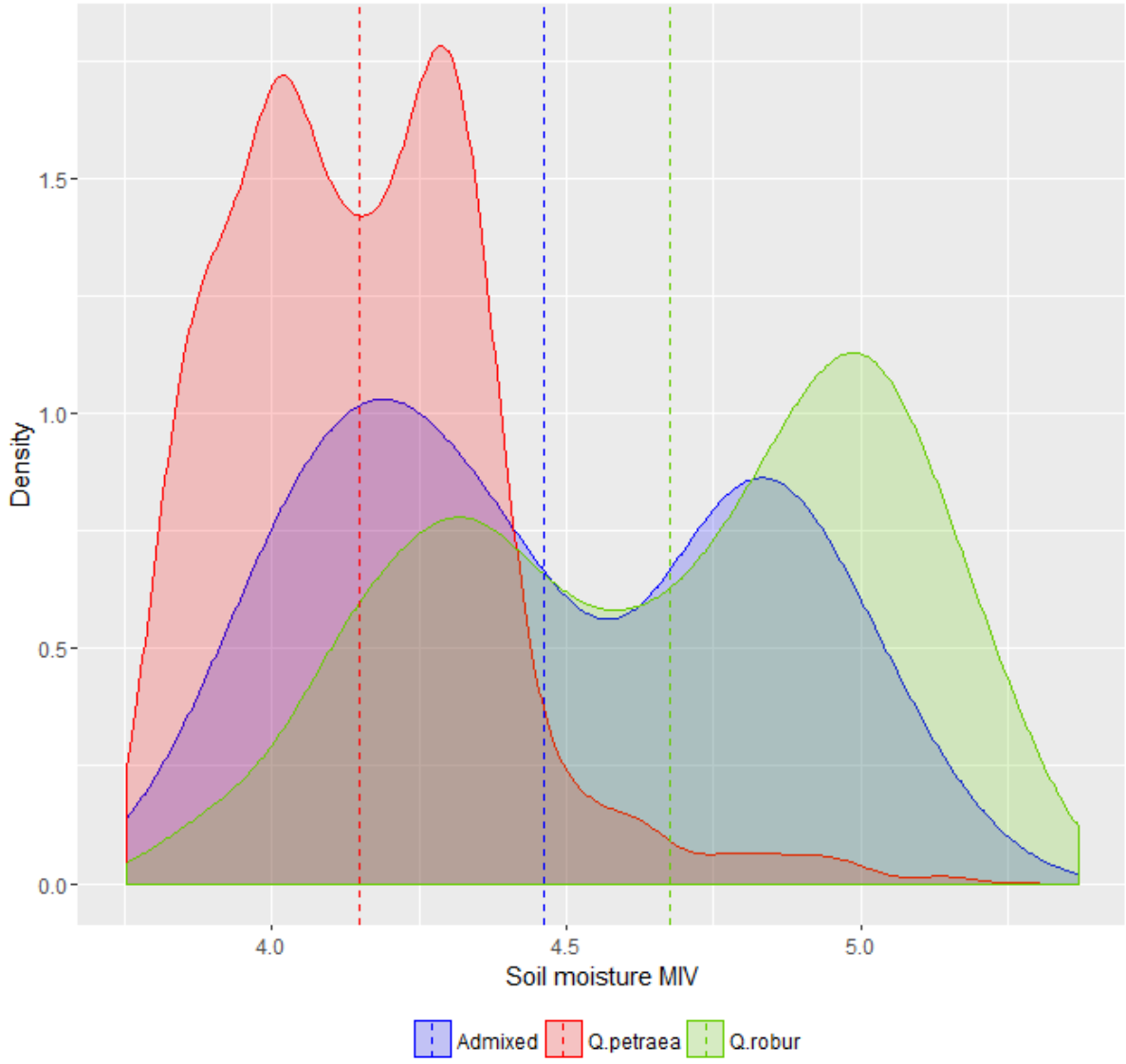


Fig. 5 Density distribution of ecological indicator values in the two species and admixed group of cohort 3

Fig.5a pH



833 **Fig. 5b** Soil moisture



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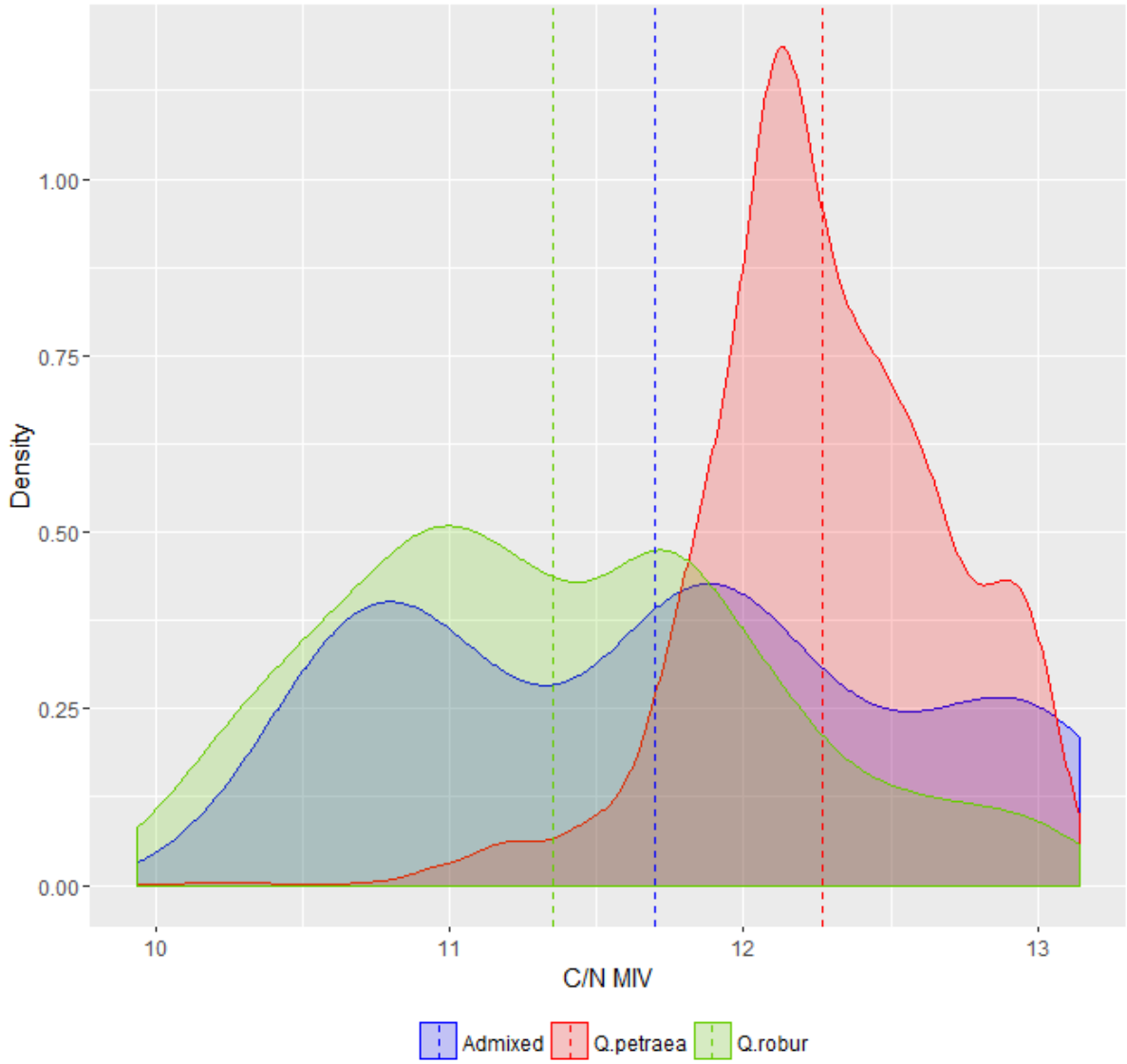
837 **Fig. 5c** Carbon/nitrogen ratio



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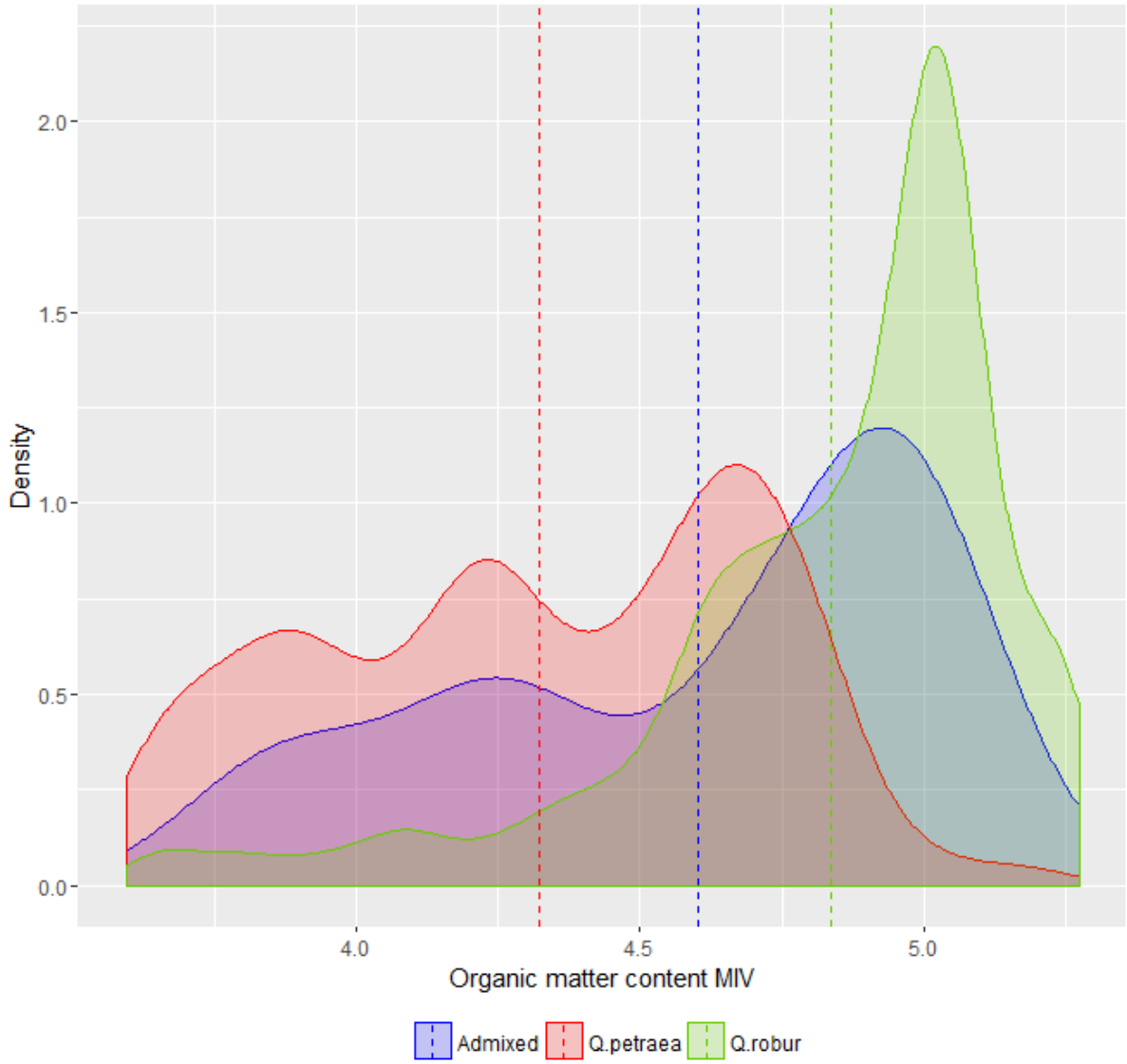
841 **Fig. 5d** Organic matter



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