

## RESEARCH ARTICLE

# Anti-herbivore defences and insect herbivory: Interactive effects of drought and tree neighbours

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## Abstract

1. How much a plant is attacked by insect herbivores likely depends on its apparency and ability to produce defensive traits, which may be modified by neighbouring plants and abiotic conditions. Yet, how much the direct and trait-mediated effects of neighbours on herbivory is modified by abiotic factors is still unknown.
2. By using a tree diversity experiment in SW France, we measured leaf insect herbivory (chewers and miners), nutritional quality (water content, C/N ratio, sugar and starch content) and chemical defences (total polyphenolics and condensed tannins) on birch (*Betula pendula*) trees growing in monocultures and mixtures with oak, pine or both species. We alleviated water stress by irrigating trees in half of the plots while trees remained unwatered (i.e. drought-stressed) in the other half.
3. Overall, insect herbivory was higher among heterospecific neighbours, which corresponds to associational susceptibility. Consistent with this finding, leaves had lower amount of anti-herbivore defences among heterospecific neighbours. In turn, insects caused more damage in drought-stressed conditions, but such effect was independent of leaf chemistry. We also found that the effect of tree species diversity on herbivory was contingent of drought conditions as associational susceptibility only occurred in drought-stressed trees. The independent and interactive effects of neighbour diversity and irrigation on leaf herbivory remained significant after accounting for birch apparency and leaf chemistry, suggesting that unmeasured plant traits or some other mechanisms not associated with plant trait variation and apparency might be involved in the observed herbivory patterns.
4. *Synthesis.* By demonstrating that associational effects are contingent upon abiotic constraints, we bring new insights into our understanding of the mechanisms driving diversity–resistance relationships across climatic gradients.

## KEYWORDS

associational effects, *Betula pendula*, biodiversity, chewers, forest, insect herbivory, miners, ORPHEE experiment

## 1 | INTRODUCTION

Ecological research conducted over the last two decades has shown that plant species diversity has substantial effects on ecosystem processes such as productivity (Cardinale et al., 2011), and also influence arthropod community structure and overall species richness at higher trophic levels (Barbosa et al., 2009; Cardinale et al., 2011; Moreira, Abdala-Roberts, Rasmann, Castagneyrol, & Mooney, 2016; Scherber et al., 2010). In particular, it is well-established that a greater diversity of plant species reduces herbivore attack by decreasing the concentration (i.e. number of individuals) and frequency (i.e. relative abundance) of herbivores' hosts ("the resource concentration hypothesis," Hambäck, Inouye, Andersson, & Underwood, 2014; Root, 1973; Underwood, Inouye, & Hambäck, 2014), or by decreasing plant apparency through an interference with physical and chemical cues used by insect herbivores to detect and reach their host plant (Castagneyrol, Giffard, Péré, & Jactel, 2013; Jactel, Birgersson, Andersson, & Schlyter, 2011). In addition, a growing number of studies has reported that plant species diversity can indirectly affect herbivory on a focal plant by modifying its nutritional quality and anti-herbivore defences (e.g. physical traits and secondary metabolites, Kostenko, Mulder, Courbois, & Bezemer, 2017; Moreira, Abdala-Roberts, Parra-Tabla, & Mooney, 2014; Mraja, Unsicker, Reichelt, Gershenson, & Roscher, 2011), independently of resource concentration and host-plant apparency. However, observed patterns are highly inconsistent and the mechanisms underlying such patterns are poorly understood. For example, some studies have reported increased investment in anti-herbivore defences among heterospecific neighbours (Moreira et al., 2014; Mraja et al., 2011), while others have reported the opposite (Glassmire et al., 2016; Kostenko et al., 2017), generating a negative (Glassmire et al., 2016) or a neutral effect (Kos, Bukovinszky, Mulder, & Bezemer, 2015; Moreira et al., 2014) on insect herbivores.

A likely source of inconsistencies among studies addressing plant diversity effects on plant-herbivore interactions is the lack of consideration of abiotic factors. It is well known that abiotic stresses such as drought directly and indirectly alter plant-herbivore interactions (Huberty & Denno, 2004; Jactel et al., 2012; Walter et al., 2011). Herbivory consumption rates and fitness, as well as insect herbivore performance, may drastically increase with increased drought stress because drought may indirectly modify plant nutritional quality and investment in anti-herbivore defences (Huberty & Denno, 2004; Jactel et al., 2012; Walter et al., 2011; White, 1974, 2009). In this sense, the plant stress hypothesis posits that abiotic stresses on plants such as lack of water enhance the nutritional quality of foliage for insect herbivores by altering biochemical source-sink relationships and foliar chemistry (Walter et al., 2011; White, 1974, 2009). For instance, Ximénez-Embún, Ortego, and Castañera (2016) reported an increase in amino acid and sugar concentrations in leaves of drought-stressed tomato plants, resulting in increased performance of the mite *Tetranychus evansi*.

To date, theory on plant diversity and drought effects on plant-herbivore interactions have been developed independently for the

most part, but recent studies addressing the effect of abiotic factors on associational resistance suggest that there are important insights to be gained from considering the two drivers together (Glassmire et al., 2016; Grettenberger & Tooker, 2016; Kambach, Kühn, Castagneyrol, & Bruelheide, 2016; Walter et al., 2011). Indeed, it is increasingly acknowledged that plant neighbourhood composition can modulate the way plants respond to water stress (Forrester, Theiveyanathan, Collopy, & Marcar, 2010; Klaus et al., 2016; Otieno et al., 2012). For instance, in accordance with the stress gradient hypothesis predicting reduced competition and increased facilitation under high stress (Callaway & Walker, 1997), Forey et al. (2016) found that beech trees growing in mixed stands increase soil nutrient acquisition in comparison to those trees growing in monospecific stands, in particular in water-stressed soils. It is therefore likely that the effects of plant diversity on leaf quality and resulting herbivory are modified by drought (Grettenberger & Tooker, 2016; Walter et al., 2011).

In the present study, we tested for independent and interactive effects of tree species diversity and drought on insect leaf herbivory (miners and chewers) and leaf chemical traits associated with defence and nutritional quality (phenolic compounds, C:N ratio, water content and carbohydrates) in silver birch trees (*Betula pendula*, Betulaceae), a common broadleaved species in the study area. In addition, we investigated if the measured tree traits, as well as tree apparency (measured as how much individual birches were taller than their neighbours, e.g. Castagneyrol et al., 2013), underlied the effects of neighbourhood diversity and drought on leaf herbivory in birch trees. To test for these effects, we used a factorial field experiment where we manipulated both host-plant species diversity (three levels: birch monocultures, two-species mixtures associating birch with the pedunculate oak *Quercus robur* or maritime pine *Pinus pinaster*, and three-species mixture with pedunculate oak, the maritime pine and birch) and water availability (two levels: irrigated vs. non-irrigated). By addressing these goals, this study builds towards a better mechanistic understanding of the combined effects of plant species diversity and abiotic conditions on plant-herbivore interactions and plant traits associated with resistance to herbivory.

## 2 | MATERIALS AND METHODS

### 2.1 | Natural history

Silver birch (*B. pendula*, Roth, Betulaceae) is a broadleaved woody species with wide climatic and edaphic tolerance. Its distribution range covers all Europe (Atkinson, 1992). Being a common pioneer species in northern and western Europe, the silver birch is naturally present in the study area where it is frequently found in association with maritime pine and various oak species. *Betula pendula* is intolerant to drought (Atkinson, 1992) which causes premature leaf drop in summer (B. Castagneyrol, pers. obs.). *Betula pendula* is attacked by a large community of insect herbivores, especially leaf-chewers and miners such as *Operophtera fagata*, *Hemichroa crocea* or *Eriocrania* spp. (Atkinson, 1992; Kunca, Csoka, & Zubrik, 2013). Even at low

damage levels, these herbivores can cause significant growth loss on birch (Zvereva, Zverev, & Kozlov, 2012).

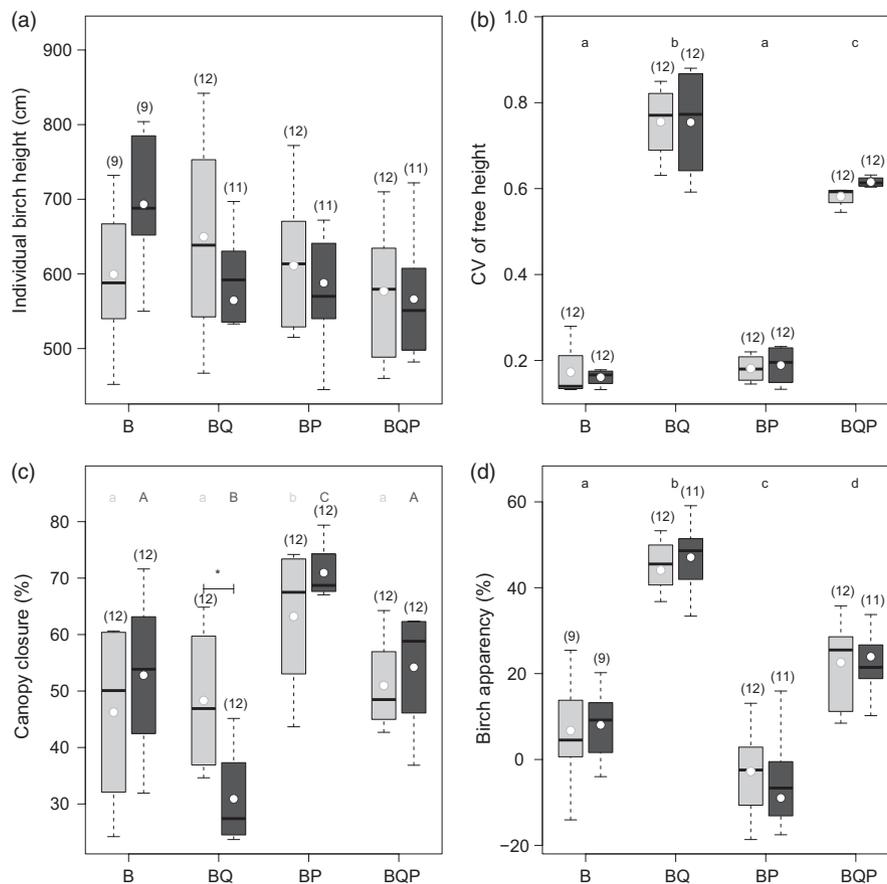
## 2.2 | Experimental design

### 2.2.1 | Tree diversity experiment

We conducted the present study at the ORPHEE Tree Diversity Experiment (<https://sites.google.com/view/orpheexperiment>) in SW France (44°44'N, 00°46'W), a long-term experiment designed to test the effects of tree species diversity on productivity and associated faunas (see Castagnéyrol et al., 2013 for a representation of the experimental design). The experiment was established in 2008 on a sandy soil (podzol) formerly planted with maritime pine. It consists of eight blocks covering 12 ha, with 32 plots in every block corresponding to the 31 possible combinations of one to five tree species (*B. pendula*, *Q. robur*, *Quercus pyrenaica*, *Quercus ilex* and

*P. pinaster*), with an additional replicate of the five-species plot. Each plot is 400 m<sup>2</sup> (20 × 20 m) and contains 10 rows of 10 trees planted 2 m apart (100 trees). Tree species mixtures were established according to a substitutive design, keeping tree density equal across plots, with equal proportion of each tree species. Within plots, individual trees from different species were planted in a regular alternate pattern, such that a tree from a given species had at least one neighbour from each of the other species.

In the present study, we focused on birch monocultures, two-species mixtures associating birch with the pedunculate oak *Q. robur* or the maritime pine *P. pinaster*, and the mixtures of pedunculate oak, maritime pine and birch, for a total of four compositions. In total, for this study we used 32 plots, corresponding to 4 plots × 8 blocks. These plots were chosen in order to systematically associate birch with a broadleaved and a conifer species and to span a large gradient of variability in tree height, canopy closure and birch apparency (Figure 1). Birch apparency represents how much (%) an individual



**FIGURE 1** Comparison of mean birch height (a) variability of tree height (b) plot canopy closure (c) and birch apparency (d) between treatments. (a) Height of the three randomly selected birches per plot at the beginning of the 2016 growing season. (b) Coefficient of variation of height of the 36 innermost trees per plot at the beginning of the 2016 growing season. (c) Canopy closure estimated in early June. (d) Apparency quantifies how much birches were on average taller (positive values) or smaller (negative values) than their neighbours (Damien et al., 2016). In all plates, light and dark greys are for control and irrigated plots respectively. B, Birch monocultures; BQ, Birch–Oak mixtures; BP, Birch–Pine mixtures; BQP, Birch–Oak–Pine mixtures. Boxes indicate the first and third quartiles. Dots and thick horizontal lines are for mean and median respectively. Contrasts among diversity and composition levels were estimated for each level of irrigation separately. Same letters above bars indicate non-significant differences among compositions. Lower case and upper case letters are for non-irrigated and irrigated treatments respectively. The effect of irrigation was tested within each level of diversity and composition, separately, and its significance is indicated by stars

birch is taller or smaller than its neighbours (Castagneyrol et al., 2013) and was calculated as  $100 \times (H_{\text{birch}} - H_{\text{plot}})/H_{\text{plot}}$ , where  $H_{\text{birch}}$  represented the height of the focal birch, and  $H_{\text{plot}}$  the mean tree height of the corresponding plot (averaged across the 36 innermost trees). Negative and positive values indicate that focal birches were on average smaller and taller than their neighbours. Canopy closure was estimated in early June as the mean percentage of pixels occupied by canopy on photos. We took four photos per plot at 1.5 m from the ground with smartphones with 8 Mpx resolution. Images were analysed with the CanopyDigi software (Goodenough & Goodenough, 2012) and percentage canopy closure was averaged at the plot level. Birch height (mean and CV), canopy closure and birch apparency were all strongly correlated with each other (Figure S1).

## 2.2.2 | Irrigation treatments

From May to October 2015 and 2016, half of the blocks were irrigated by sprinkling c. 42 m<sup>3</sup> per night and per block, corresponding to c. 3 mm per night per plot. This volume was calculated based on regional climatic data (evapotranspiration). We supplied 60% of yearly precipitations during the five summer months (Figure S2), which enabled to avoid any soil water deficit in the irrigated blocks during the entire growing season.

We used three approaches to confirm that water stress was alleviated in irrigated blocks (Figure S3, Castagneyrol et al., 2017). First, we quantified soil gravimetric water content in late August 2016 by sampling two soil cores between 20 and 40 cm in each of the selected 32 plots. We pooled the two samples in hermetic plastic bags and stored them into portable fridges before being brought to the laboratory where samples were immediately weighted. We dried samples at 105°C for 72 hr and weighted them. Gravimetric water content was estimated as the proportion of water in fresh samples. Second, we measured predawn leaf water potential ( $\psi_w$ ) in early September 2016 on one birch taken close to the centre of each studied plot.  $\psi_w$  was used as a proxy of the spatially integrated water potential of the soil explored by roots. We used a Scholander-type pressure chamber (DG meca, type NP100, model 0-100bars) to measure  $\psi_w$  on one leaf per tree. Leaves were sampled after sap pressure re-equilibrated, between 4.30 and 6.30 a.m. local time (before sunrise) with clippers connected to extension loppers. Third, we compared birch relative growth rate (RGR) between irrigated and non-irrigated plots. In early 2015 ( $C_{2015}$ ) and early 2016 ( $C_{2016}$ ), we measured tree circumference at 1.30 m from ground level on a random sample of seven birches per plot (provided they were tall enough), in all irrigated and non-irrigated plots. This resulted in 852 measurements. RGR was estimated as the percentage of circumference increment ( $100 \times (C_{2016} - C_{2015})/C_{2015}$ ) during the first year of irrigation and was used to confirm that birches integrated the effect of irrigation through the whole growing season.

In France, summer 2016 was one of the driest of the 1959–2016 period ([www.meteofrance.fr](http://www.meteofrance.fr)). During the 2016 growing season, in addition to natural precipitations, irrigation brought c. 550 mm water per plot, which represents half of total precipitations received

that year (Figure S2). In the absence of irrigation, the water-table was 50 cm below-ground level in early season and went down to -1.50 m in October. In September 2016, soil water content between 20 and 40 cm was 2.6 times higher in irrigated plots than in non-irrigated plots ( $F_{1,30} = 26.22$ ,  $p < .001$ , Figure S3). Soil water content was independent of tree species composition ( $F_{3,27} = 1.50$ ,  $p = .237$ , Figure S3) or composition  $\times$  irrigation interaction ( $F_{3,24} = 2.22$ ,  $p = .112$ , Figure S3). Birch  $\psi_w$  was 3.8 times lower (i.e. more water stressed) in control plots ( $M \pm SE$ :  $-1.63 \pm 0.08$  MPa) than in irrigated plots ( $-0.43 \pm 0.09$  MPa). Together with the observation that birch growth rate was higher in irrigated than non-irrigated plots ( $F_{1,5,6789} = 26.64$ ,  $p = .003$ , Figure S3), these results confirm that irrigation created two contrasted treatments, one in which trees were water stressed and another one in which water was not limiting.

## 2.3 | Measurements of insect herbivory

We measured damage caused by two guilds of leaf insect herbivores: chewers and miners. Within the selected 32 plots (4 plots  $\times$  8 blocks) we randomly sampled three individual birch trees out of the 36 innermost trees of each plot (resulting in a total of 96 birches). Damage caused by the two guilds was assessed twice to test for a season effect, in early June (early summer in the study area) and late July 2016 (midsummer in the study area) by visual inspection of 50 leaves per sampled birch (Johnson, Bertrand, & Turcotte, 2016). Because of possible vertical stratification of attacks within canopy, we randomly selected leaves on four randomly chosen branches, two at 2 m and two at 5 m high.

The percentage of leaf area removed (LAR) by leaf-chewers was estimated on each sampled leaf by a unique observer all along the experiment (BC) using seven classes (0%, 1%–5%, 6%–15%, 16%–25%, 26%–50%, 51%–75% and >75% LAR) and then averaged per sampled tree using the mid-point of each damage class (e.g. Castagneyrol et al., 2013). Leaf-miner damage was measured as the proportion of leaves with at least one mine (leaf-miner incidence henceforth; Barton et al., 2014; Kozlov, Skoracka, Zverev, Lewandowski, & Zvereva, 2016). While chewing damage could be made by either generalist or specialist herbivores, leaf miners found on birch were strictly specialist herbivores in the study area.

## 2.4 | Chemical analyses

We quantified constitutive total polyphenolic and condensed tannins content as proxies for chemical defences. We quantified constitutive leaf dry matter content (LDMC), C:N ratio, starch and soluble sugar concentrations as proxies for leaf nutritional quality. These constitutive plant traits are widely recognized as herbivore feeding stimulant and deterrents and to confer resistance against insect herbivores in many plant taxa (Abdala-Roberts et al., 2016; Forkner, Marquis, & Lill, 2004; Loranger et al., 2013; Pearse, 2011; Schoonhoven, 2005).

In late July 2016, we collected five to six undamaged leaves in each of the 96 sampled trees. Leaves were directly put into

hermetic plastic bags filled with humidified absorbent paper. They were stored at dark into portable fridge before being brought back to the laboratory where they were kept at 4°C to rehydrate for 48 hr, at dark (Pérez-Harguindeguy et al., 2013). Leaves were then dried for 48 hr at 55°C and weighted again to estimate LDMC ( $\text{mg}_{\text{dry}}/\text{g}_{\text{fresh}}$ ; Pérez-Harguindeguy et al., 2013). Then, leaves were finely grounded with liquid nitrogen to quantify the concentration of constitutive total polyphenolics, condensed tannins, starch, soluble sugars and C:N ratio. Low LDMC, C:N ratio and concentrations of polyphenolics and condensed tannins on the one hand and high concentrations of sugars and starch on the other hand are commonly associated with good leaf quality to herbivores (Abdala-Roberts et al., 2016; Forkner et al., 2004; Loranger et al., 2013; Pearse, 2011; Schoonhoven, 2005).

Leaf total phenolics were extracted and analysed as described by Moreira, Mooney, Zas, and Sampedro (2012) and Moreira, Zas, and Sampedro (2012). Briefly, they were extracted from 20 mg of plant tissue with 70% methanol in an ultrasonic bath for 15 min, followed by centrifugation and subsequent dilution of the methanolic extract. Total phenolic content was determined colorimetrically by the Folin-Ciocalteu method in a Biorad 650 microplate reader (Bio-Rad Laboratories, PA, USA) at 740 nm, using tannic acid as standard. Condensed tannins in the aqueous methanol extracts were determined by the procyanidine method as in Sampedro, Moreira, and Zas (2011). The methanolic extract was mixed with acidified butanol and a ferric ammonium sulphate solution, allowed to react in a boiling water bath for 50 min and then cooled rapidly on ice. The concentration of condensed tannins in this solution was determined colorimetrically in a Biorad 650 microplate reader at 550 nm, using as standard purified condensed tannins of quebracho (*Schinopsis balansae* Engl.; Droguería Moderna, Vigo, Spain). We expressed phenolic compound concentrations in mg/g tissue on a dry weight basis.

The concentrations of soluble sugars and nonstructural carbohydrate reserves (starch) in the leaves were analysed by the anthrone method (Hansen & Møller, 1975). Soluble sugars were extracted from finely grounded leaves (50 mg) with aqueous ethanol (80% v/v). Starch was extracted with 1.1% hydrochloric acid in a water bath at 100°C for 30 min, followed by centrifugation and subsequent dilution of the extract. Soluble sugars and starch concentration were determined colorimetrically in a Biorad 650 microplate reader at 630 nm, using glucose and potato starch, respectively, as standards.

Leaf C content (%) and total foliar N content (%) were measured with an Elemental Analyser/Gas Analyser - Isotope-Ratio Mass Spectrometry (Carlo Erba, Elementar, Finnigan, Isoprime, Bremen, Germany).

## 2.5 | Statistical analyses

### 2.5.1 | General modelling approach

The ORPHEE experiment followed a randomized split-plot design replicated in eight blocks, with *Irrigation* (four replicates of two levels: irrigated vs. non-irrigated, with irrigation homogenizing hydric

conditions among plots and across time) as the whole factor and neighbour identity (henceforth *Neighbours*, four levels: monocultures of birch, two-two-species mixtures including birch with pine or oak trees, and one-three-species mixtures of birch, oak and pine trees) as the split factor. The split-plot design requires adapting the calculation of degrees of freedom and mean sum of squares of residuals (Altman & Krzywinski, 2015). This was achieved using linear mixed effect models (LMM), with *Block* and *Block* × *Irrigation* as random factors (1|*Block:Irrigation* in R syntax). For leaf miner data, we used Generalized linear mixed effect models (GLMM) with binomial error distribution and *logit*-link. Herbivory and leaf trait data were analysed at the tree level. Non-independence of individual birches within plots was accounted for by defining *Plot* as a random factor, nested within the *Block* factor (Schielzeth & Nakagawa, 2013).

For each test (see below), we first built a full model including all fixed effects and their interactions. The full model was then simplified by sequentially dropping non-significant terms, starting with highest-order interactions. Model parameters were finally estimated on the most parsimonious obtained model, using restricted maximum likelihood for LMM. We estimated marginal ( $R^2_m$ ) and conditional ( $R^2_c$ )  $R^2$  following Nakagawa and Schielzeth (2013). Whenever necessary, response variables were log-transformed to meet model assumptions.

All analyses were done in R version 3.3.1 (R Core Team, 2016), using the following packages: *lmerTest*, *car*, *multcomp*, *car* and *MuMIn* (Bartoń, 2016; Fox et al., 2016; Hothorn et al., 2016; Kuznetsova, Brockhoff, & Christensen, 2015).

### 2.5.2 | Insect herbivory

Insect herbivory was measured in early June (early season) and late July (mid season). We first built a full LMM including *Season*, *Irrigation* and *Diversity* (i.e. monoculture vs. mixture) or *Neighbours* as explanatory factors, and all two- and three-ways interactions. We added individual tree identity nested within *Plot* factor as a random factor to account for variance arising for repeated measurements on the same individual trees.

### 2.5.3 | Plant defensive traits

We first analysed all leaf traits separately using the same modelling approach, with birch apparency, tree species diversity or neighbour identity, irrigation and *Irrigation* × *Diversity* (or *Irrigation* × *Neighbours*) and *Irrigation* × *Apparency* interactions as fixed effects in LMM.

Because some leaf traits are highly correlated (Figure S4), we further used a PCA to summarize information contained in the six leaf traits. We extracted coordinates on the two-first PC axes (PC1 and PC2, respectively) and tested whether they were predicted by tree height, irrigation and composition using the same LMM described above. The first and second principal component (PC) axis of the multivariate principal component analysis explained 44.3% (PC1) and 18.3% (PC2) of variability in leaf traits respectively. PC1 was driven by chemical defences, positive values being associated with

high concentrations of both total polyphenolics and condensed tannins (Figure 3a). PC2 was driven by nutritional quality related traits. Positive values were associated with high LDMC and C:N content (i.e. low water and low N content), while negative values corresponded to high concentrations of starch and soluble sugars (Figure 3a). Negative values therefore indicate greater nutritional leaf quality.

### 2.5.4 | Plant traits underlying effects of irrigation and tree diversity or neighbour identity on leaf herbivory

We finally used a multiple regression approach and included leaf traits (PC1, PC2 and PC1  $\times$  PC2 interaction) and birch apparency into a full model testing the effects of irrigation and tree species diversity or neighbour identity on leaf herbivory. This was limited to mid season data, when traits were measured. We then applied the simplification procedure explained above. Because birch apparency (Figure 1) and leaf traits (see Section 3) were not independent of tree diversity or neighbour identity, the multiple regression approach allows testing whether some variance remained to be explained by the latter when accounting for the former, and conversely. We expected that if defences mediate effects of irrigation and tree diversity on leaf herbivory, then significant effects of any of these factors (or their interaction) should become non-significant once such traits are accounted for in the model. If irrigation and tree diversity effects remain significant after including these traits, this suggests that irrigation and tree diversity influence herbivory through other unmeasured plant traits or via some other mechanism not associated with plant trait variation.

## 3 | RESULTS

### 3.1 | Tree apparency

Tree apparency was strongly determined by the specific composition of experimental plots (Figure 1), which reflected variability in tree height at the plot scale (Figure 1). Notably, birch apparency was the highest in birch–oak mixtures (Figure 1) as a result of the smaller size of oaks compared to birches (see figure 2 in Damien et al., 2016). At the opposite, birch apparency was minimal in birch–pine mixtures (Figure 1) where birches were partially hidden by taller pine neighbours. Birch apparency correlated with other stand-level variables describing tree growth and absolute and relative height (Figure S1). Henceforth, we focused on apparency instead of any other variables as it integrates information about both the focal birches and their neighbours height.

### 3.2 | Leaf insect herbivory

#### 3.2.1 | Leaf-chewer damage

Leaf-chewer damage ranged from 0.34% to 15.80% (mean (*M*): 4.08  $\pm$  2.31%). The season of sampling, plant species diversity and

irrigation treatment significantly affected leaf-chewer damage (Table 2). Specifically, leaf-chewer damage was 1.5 times greater in midsummer than in early summer. It was significantly higher in mixtures than in monoculture plots and it was 1.5 times higher in non-irrigated than in irrigated plots (Figure 2). Associational effects depended on irrigation treatment as demonstrated by the significant *Diversity*  $\times$  *Irrigation* and *Neighbour*  $\times$  *Irrigation* interactions (Table 1). The effects of diversity and composition on leaf-chewer damage were significant only in non-irrigated plots where leaf-chewer damage was greater in species mixtures than in species monocultures (Figure 2). Leaf-chewer damage was higher in birch–oak mixtures, lower in monocultures and intermediate in mixtures containing pine (Figure 2). We did not find significant effects of *Season*  $\times$  *Diversity*, *Season*  $\times$  *Neighbour* or *Season*  $\times$  *Irrigation* interactions on leaf-chewer damage (Table 1).

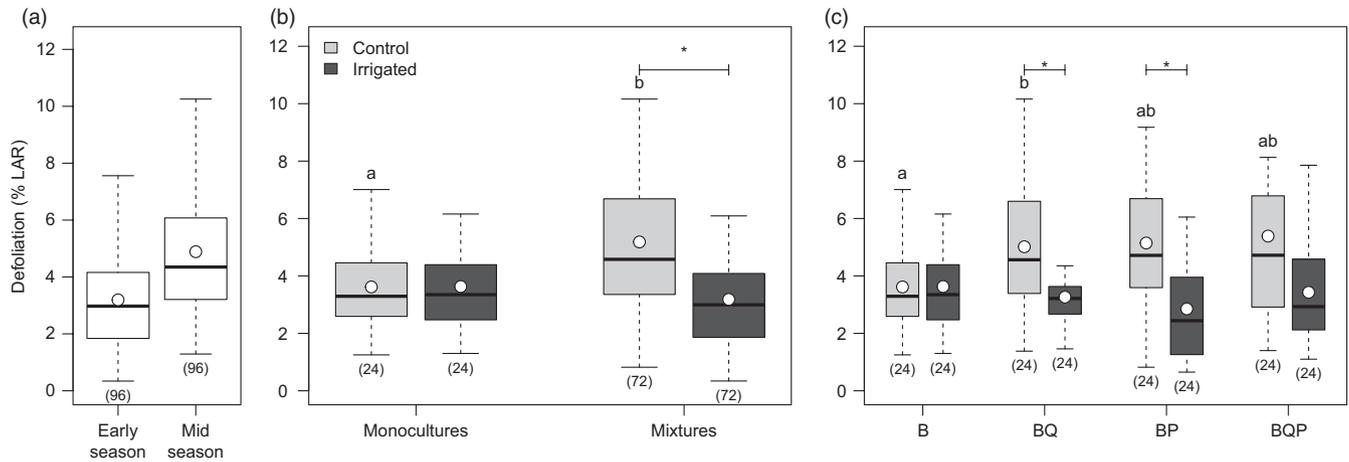
#### 3.2.2 | Leaf-miner incidence

Leaf-miner incidence (% leaves with at least one mine) ranged from 0% to 24% (mean (*M*): 5.00  $\pm$  4.26%). The season of sampling and plant species diversity significantly affected leaf-miner incidence (Table 2). Specifically, leaf-miner incidence was 1.5 times greater in midsummer than in early summer and 1.5 times higher in mixtures than in monoculture plots (i.e. associational susceptibility) and 1.3 times higher in control than in irrigated plots (Figure 3).

There was a significant effect of the *Season*  $\times$  *Diversity*  $\times$  *Irrigation* ( $\chi^2 = 11.48$ , *df* = 1, *p* < .001) and *Season*  $\times$  *Diversity*  $\times$  *Neighbour* ( $\chi^2 = 17.76$ , *df* = 3, *p* = .006) three-ways interactions on leaf-miner incidence such that early and mid season data were analysed separately (Table 1). Overall, in both seasons, leaf-miner incidence was lower in monocultures, intermediate in two-species mixtures and higher in three-species mixtures (i.e. associational susceptibility). However, in details, this effect depended on irrigation treatment as demonstrated by the significant *Diversity*  $\times$  *Irrigation* and *Neighbour*  $\times$  *Irrigation* interactions (Table 1). In early season, associational susceptibility only occurred in non-irrigated plots while there was no effect of tree diversity or identity in irrigated plots (Figure 3). At the contrary, in late season, associational susceptibility only occurred in irrigated while leaf-miner incidence did not differ significantly among composition treatments in non-irrigated plots (Figure 3).

### 3.3 | Plant defensive traits

Tree species diversity (i.e. monocultures vs. mixtures) and neighbour identity (i.e. birch monocultures, birch–oak, birch–pine and birch–oak–pine mixtures) had qualitatively similar effects on leaf traits (Table S1). Concentration of total polyphenolics was significantly 1.2-fold higher in trees growing in monocultures than in trees growing in mixtures (Table S1, Figure S5). Specifically, the highest concentration of total polyphenolics was found in trees growing in monocultures, the lowest in trees growing in three-species mixtures, and intermediate in trees growing in two-species mixtures (Table 2). None of other traits, individually, responded to tree species diversity or neighbour identity (Table 2, Table S1, Figure S5).



**FIGURE 2** Effects of season (a), diversity (b) and plot specific composition (c) and irrigation on leaf-chewer damage. In (b) and (c), contrasts among compositions were estimated for each level of irrigation separately. Same letters above bars indicate non-significant differences among compositions. The effect of irrigation was tested within each level of diversity and composition, separately, and its significance is indicated by stars. Legends are the same as in Figure 1

**TABLE 1** Summary of LMM (for leaf-chewers) and GLMM (for leaf-miners) testing the effects of irrigation, and tree species diversity (monoculture vs. mixture) or neighbour identity (birch, oak, pine, oak + pine) on leaf-chewer damage and leaf-miner incidence

Season	Predictors	Leaf-chewers			Leaf-miners		
		<i>F</i> ( <i>df</i> )	<i>p</i> -value	$R^2_m$ ( $R^2_c$ )	$\chi^2$ ( <i>df</i> )	<i>p</i> -value	$R^2_m$ ( $R^2_c$ )
Early	Irrigation	3.50 (1, 92)	.065	0.14 (0.14)	1.54 (1)	.215	0.05 (0.07)
	Diversity	0.04 (1, 92)	.847		1.50 (1)	.220	
	Irrigation × Diversity	4.06 (1, 92)	<b>.047</b>		10.40 (1)	<b>.001</b>	
Mid	Irrigation	3.76 (1, 8.69)	.086	0.25 (0.46)	0.90 (1)	.342	0.02 (0.07)
	Diversity	1.87 (1, 22)	.185		4.26 (1)	<b>.039</b>	
	Irrigation × Diversity	5.08 (1, 22)	<b>.035</b>		0.44 (1)	.509	
Early	Irrigation	11.55 (1, 88)	<b>.001</b>	0.20 (0.20)	2.02 (1)	.156	0.05 (0.07)
	Neighbours	1.22 (3, 88)	.306		5.97 (3)	.113	
	Irrigation × Neighbours	2.93 (3, 88)	<b>.038</b>		13.38 (3)	<b>.004</b>	
Mid	Irrigation	10.03 (1, 6)	<b>.019</b>	0.18 (0.45)	0.94 (1)	.333	0.03 (0.07)
	Neighbours	1.59 (3, 21)	.223		8.06 (3)	<b>.045</b>	
	Irrigation × Neighbours	2.08 (3, 18)	.138		8.52 (3)	<b>.036</b>	

LMM, linear mixed effect models; GLMM, Generalized linear mixed effect models. Although for leaf-chewers the three-ways Season × Irrigation × Diversity and Season × Irrigation × Neighbours interactions were not significant, we report results for early and mid season separately to be consistent with leaf-miners and to show that the interactive effects of irrigation and neighbour diversity or identity were consistent across seasons. Bold characters indicate significant predictors at  $\alpha = 0.05$ .  $R^2$  were reported for the simplified model (i.e. after the interaction was removed if not significant).  $R^2_m$  and  $R^2_c$  correspond to marginal (i.e. accounting for fixed effects only) and conditional (accounting for both fixed and random effects)  $R^2$ .

Starch concentration was 1.1-fold higher in trees growing in non-irrigated plots than in trees growing in irrigated plots (Figure S5, Table 2). Individually, none of other traits were significantly affected by irrigation (Table 2). Finally, LDMC was the only trait being significantly influenced by birch apparency and was significantly higher in more apparent birches (slope  $\pm$  SE:  $4.1 \times 10^{-4} \pm 1.1 \times 10^{-4}$ , Table 2, Figure S5), indicating that leaves had a lower water content in more apparent than in less apparent birches.

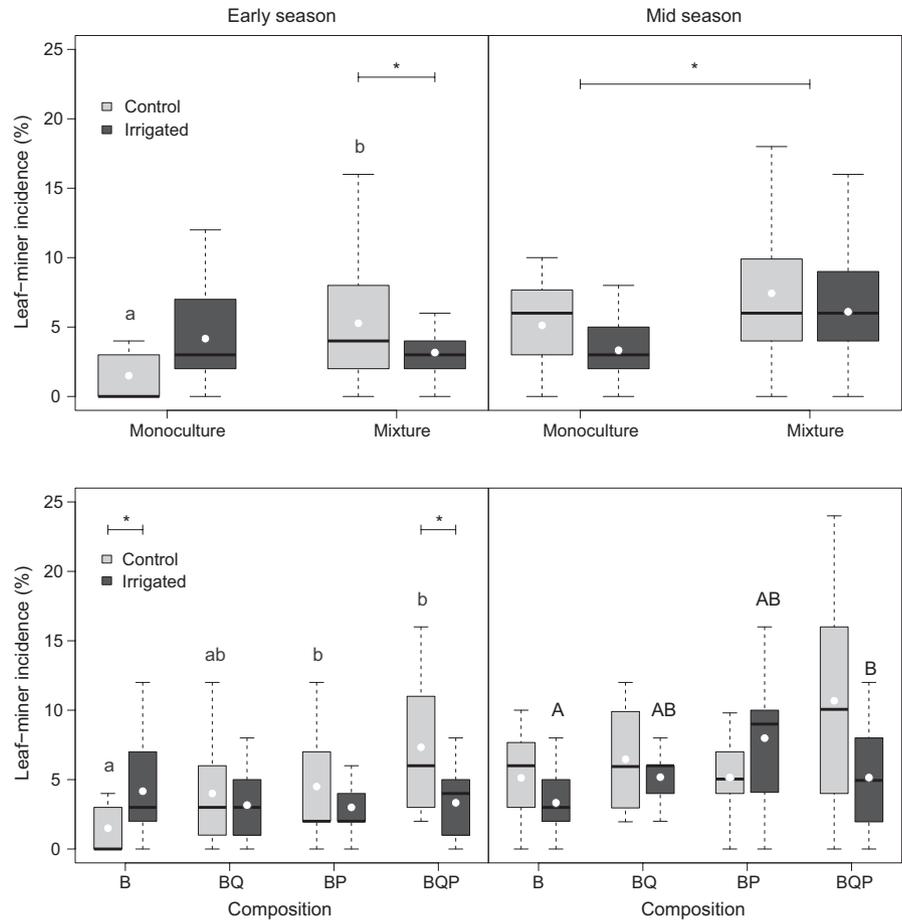
There was a significant effect of tree species diversity and neighbour identity on PC1 (Table 2, Table S1), with chemical

defences being greater in trees growing in monocultures than in trees growing in any other mixture (Figure 4b). Coordinates on PC1 increased with tree apparency (Table 2, Figure S5, slope  $\pm$  SE:  $3.6 \times 10^{-2} \pm 1.5 \times 10^{-2}$ ), indicating that investment in chemical defences increased as birches were more apparent. Despite a tendency towards higher leaf nutritional quality (i.e. leaves with higher water, sugar, starch and nitrogen content) in trees growing in non-irrigated plots (Figure 4c), there was no effect of birch apparency, irrigation or tree species diversity or neighbour identity on PC2 axis.

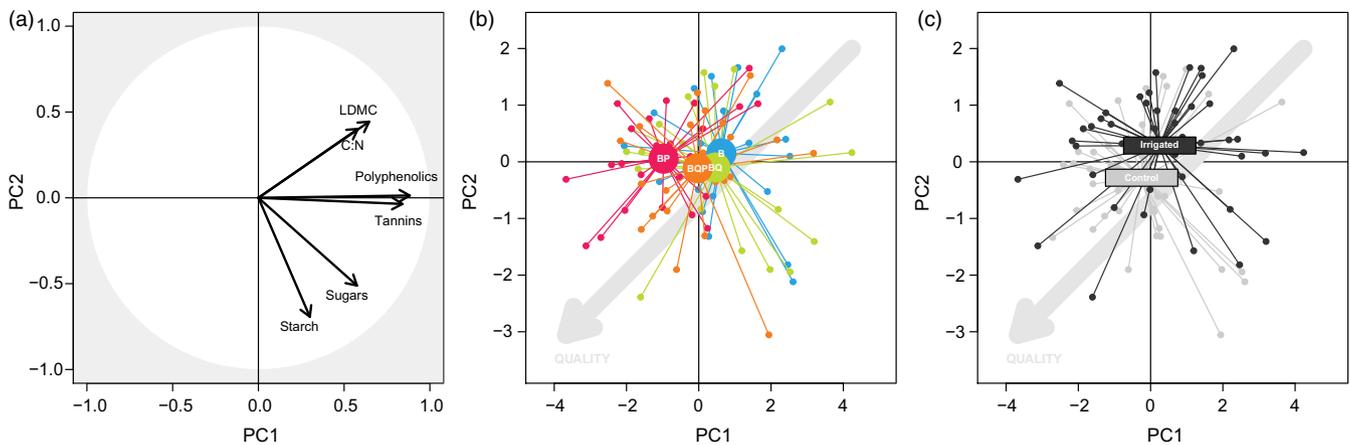
Response	Predictor	F-value (df)	p-value	$R^2_m$ ( $R^2_c$ )
LDMC	Apparency	13.67 (1, 34.1)	<b>.001</b>	0.15 (0.32)
	Neighbours	0.84 (3, 26.1)	.485	
	Irrigation	0.81 (1, 5.8)	.404	
	Apparency × Irrigation	1.03 (1, 33.5)	.318	
	Neighbours × Irrigation	0.51 (3, 23.4)	.676	
Total polyphenolics	Apparency	2.33 (1, 79)	.131	0.12 (0.37)
	Neighbours	4.20 (3, 17.8)	<b>.021</b>	
	Irrigation	0.49 (1, 5.8)	.513	
	Apparency × Irrigation	0.38 (1, 74.5)	.538	
	Neighbours × Irrigation	0.86 (3, 71.2)	.466	
Condensed tannins	Apparency	3.48 (1, 78.8)	.066	-(0.39)
	Neighbours	2.23 (3, 18)	.120	
	Irrigation	0.51 (1, 5.6)	.505	
	Apparency × Irrigation	0.18 (1, 27.1)	.672	
	Neighbours × Irrigation	0.31 (3, 20.7)	.819	
C:N	Apparency	2.02 (1, 56.4)	.160	-(0.6)
	Neighbours	1.03 (3, 26.6)	.395	
	Irrigation	1.26 (1, 6)	.304	
	Apparency × Irrigation	0.78 (1, 66)	.379	
	Neighbours × Irrigation	1.12 (3, 17.1)	.367	
Starch	Apparency	0.64 (1, 75.5)	.426	0.08 (0.46)
	Neighbours	0.88 (3, 27.4)	.462	
	Irrigation	4.30 (1, 30.4)	<b>.047</b>	
	Apparency × Irrigation	0.27 (1, 74.8)	.604	
	Neighbours × Irrigation	2.82 (3, 24.2)	.060	
Sugars	Apparency	1.42 (1, 76.1)	.238	-(0.32)
	Neighbours	1.04 (3, 21.8)	.394	
	Irrigation	0.16 (1, 6)	.702	
	Apparency × Irrigation	0.05 (1, 75.1)	.825	
	Neighbours × Irrigation	1.95 (3, 18.4)	.157	
PC1	Apparency	5.36 (1, 79.6)	<b>.023</b>	0.20 (0.38)
	Neighbours	3.45 (3, 25)	<b>.032</b>	
	Irrigation	0.85 (1, 5.5)	.395	
	Apparency × Irrigation	0.30 (1, 29.3)	.591	
	Neighbours × Irrigation	0.66 (3, 22.5)	.586	
PC2	Apparency	<0.01 (1, 73.4)	.978	-(0.32)
	Neighbours	0.16 (3, 20.6)	.923	
	Irrigation	3.92 (1, 6.1)	.094	
	Apparency × Irrigation	1.18 (1, 73.2)	.281	
	Neighbours × Irrigation	1.74 (3, 17.7)	.196	

**TABLE 2** Summary of linear mixed effect models (LMM) testing the effects of birch apparency, irrigation and tree neighbour identity on leaf nutritional quality and defensive traits

F-values, degrees of freedom (numerator, denominator) are given together with significance. Bold characters indicate significant predictors at  $\alpha = 0.05$ .  $R^2_m$  and  $R^2_c$  correspond to marginal (i.e. accounting for fixed effects only) and conditional (accounting for both fixed and random effects)  $R^2$ .  $R^2_m$  was calculated only when the final model retained at least one predictor.



**FIGURE 3** Effects of irrigation and tree species diversity (a, b) or composition (c, d) on leaf-miner incidence in early (a, c) and mid season (b, d). Contrasts among diversity and composition levels were estimated for each level of irrigation separately. Same letters above bars indicate non-significant differences among compositions. Lower case and upper case letters are for non-irrigated and irrigated treatments respectively. The effect of irrigation was tested within each level of diversity and composition, separately, and its significance is indicated by stars. Legends are the same as in Figure 1



**FIGURE 4** Multivariate analysis of leaf traits. (a) Correlation circle showing correlations among leaf traits and between leaf traits and PC axes. (b, c) Projections of individual trees on PC axes according to (b) tree species composition and (c) irrigation treatment. Thick grey arrows point towards greater leaf quality (i.e. lower defences and greater nutritional quality) [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

### 3.4 | Plant traits underlying effects of tree species diversity and irrigation on leaf herbivory

#### 3.4.1 | Leaf-chewer damage

The effects of irrigation, plant diversity or neighbour identity and their interaction on leaf chewer damage remained significant

(Irrigation × Diversity:  $F_{1,22} = 5.08, p = .035$ ; Irrigation × Neighbours:  $F_{3,75.5} = 5.54, p = .002$ ) after including birch apparency and plant defensive traits (PC1 and PC2 axes) as covariates in the statistical model. Leaf-chewer damage increased with coordinates on PC2 axis (slope ± SE:  $0.15 \pm 0.06$ ), suggesting greater consumption of leaves of low quality. This effect was, however, significant only in non-irrigated plots ( $PC2 \times Irrigation: F_{1,80.94} = 5.86, p = .017$ , Figure S6). Birch apparency had

no effect on leaf-chewer herbivory. Altogether, these results suggest that effects of irrigation and tree species diversity on chewer herbivory were not mediated by the studied plant traits nor by tree apparency.

### 3.4.2 | Leaf-miner incidence

The effects of irrigation, plant diversity and their interaction on leaf-miner incidence remained significant after including birch apparency and plant defensive traits as covariates in the statistical model ( $\chi^2 = 4.84$ ,  $df = 1$ ,  $p = .028$ ), with greater leaf-miner incidence in mixtures than in monocultures (Figure 3). This suggests that effects of irrigation and tree species diversity on leaf-miner incidence were not mediated by the studied plant traits nor by tree apparency.

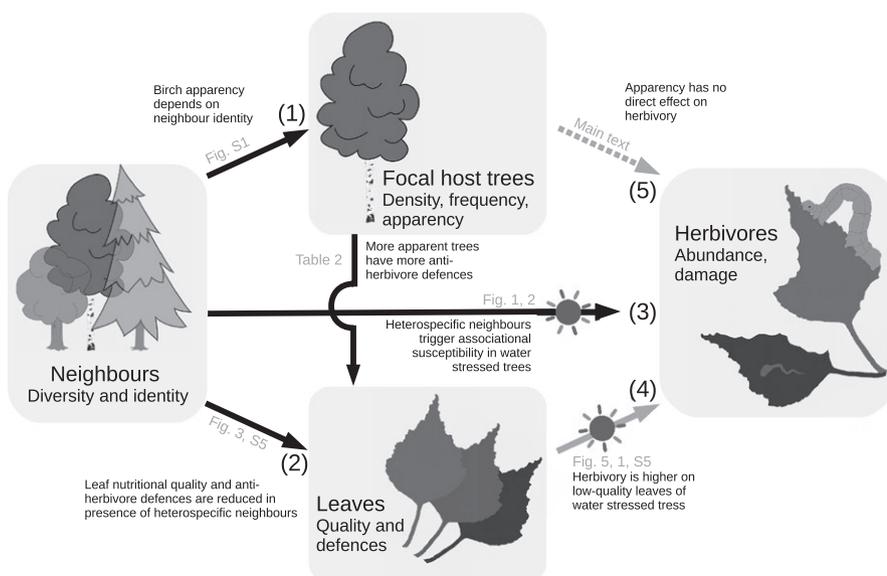
Although neither leaf traits (PC1:  $\chi^2 = 0.04$ ,  $df = 1$ ,  $p = .839$ ; PC2:  $\chi^2 = 0.06$ ,  $df = 1$ ,  $p = .799$ ) nor birch apparency ( $\chi^2 = 0.11$ ,  $df = 1$ ,  $p = .741$ ) had a significant effect on leaf-miner incidence, the effect of tree neighbour identity was no longer significant ( $\chi^2 = 6.31$ ,  $df = 3$ ,  $p = .097$ ) when introduced in the same model, suggesting that leaf traits and birch apparency could partially account for some part of variability in leaf-miner incidence among tree species compositions.

## 4 | DISCUSSION

Despite decades of independent investigations on plant diversity or drought effects on insect herbivory, results remain inconsistent and a comprehensive understanding of mechanisms at play is still missing. In this sense, our study reveals that part of such inconsistencies may result from the overlooked effects of abiotic constraints on tree diversity effects, and vice versa (see Figure 5 for a graphical summary of our results). In particular, we show that insect herbivory was higher among heterospecific neighbours than among conspecifics, which corresponds to an associational susceptibility effect, but these patterns were contingent upon abiotic environment and occurred only in water-stressed trees.

Our results revealed that the interactive effect of neighbours and drought on insect herbivory was partly influenced by neighbour-mediated changes in plant nutritional quality and production of constitutive defences. This result is consistent with previous studies showing that plant ability to defend against herbivores can be affected by abiotic conditions such as water or nutrient availability (Herms & Mattson, 1992; Jactel et al., 2012; Walter et al., 2011; White, 1974) and with more recent studies reporting that the diversity and identity of plant's neighbours can modify its production of anti-herbivore defences (Kostenko et al., 2017; Moreira, Glauser, & Abdala-Roberts, 2017; Moreira et al., 2014; Mraja et al., 2011). It is thus possible that the strength and direction of associational effects is modulated by abiotic factors (Kambach et al., 2016), partly because both contribute to control leaf nutritional quality and anti-herbivore defences.

Insect herbivory on birch was greater among heterospecific neighbours (i.e. associational susceptibility, White & Whitham, 2000; Barbosa et al., 2009). Three classical mechanisms have been proposed to explain associational susceptibility such as (1) the spill-over of herbivores from neighbouring species onto birches (White & Whitham, 2000), (2) the biased landing rate of herbivores on the most apparent host trees (Hambäck et al., 2014), (3) the concentration of specialist herbivores on the fewer birches available in mixtures (Bañuelos & Kollmann, 2011; Damien et al., 2016; Otway, Hector, & Lawton, 2005) or a combination of these three mechanisms. Because our tree diversity experiment follows a replacement design, birch concentration (i.e. number of individuals per plot) is correlated with its frequency (i.e. relative number of birches and associated species). Pure associational effects cannot be therefore separated from concentration and dilution effects (Damien et al., 2016). All these hypotheses assume that associational susceptibility results from differential colonization of host trees among different neighbours. Alternatively, (4) the dietary mixing hypothesis (Bernays, Bright, Gonzalez, & Angel, 1994; Hägele & Rowell-Rahier, 1999) proposes that associational susceptibility could be due to generalist herbivores benefiting from feeding on various resources



**FIGURE 5** Summary of results. Each pathway refers to a specific figure or table. Pathways represent our initial hypotheses. Black arrows represent strong support of our results to the pathway, whereas grey solid and dashed arrows represent partial and absence of support respectively. Suns indicate that the corresponding pathway was only observed in stressed tree

differing in nutritional quality or levels of toxicity (Lefcheck, Whalen, Davenport, Stone, & Duffy, 2013). Previous studies using the same experimental design suggested that tree apparency and host concentration hypotheses were the main factors driving insect herbivory (Castagneyrol et al., 2013, 2017; Damien et al., 2016). However, while the apparency hypothesis could explain the greater herbivory in birch-oak mixtures (birches were taller than oaks), it cannot account for herbivory in birch-pine mixtures where birch trees were less apparent (birches smaller than pines) than in pure birch stands. Our results do not support either the dietary mixing hypothesis as both leaf-chewers (likely consisting in both generalists and specialists) and leaf miners (mostly specialist species) similarly responded to tree diversity treatments. Consequently, none of the above mentioned hypotheses could individually explain our observed patterns.

By contrast, our findings partly support a fifth alternative hypothesis: (5) associational effects would result from neighbour-induced changes in leaf chemistry. In particular, birch leaves had greater amounts of constitutive defences in species monocultures than in species mixtures and this led to concomitant patterns of reduced herbivore damage in monocultures, for both chewers and miners. However, once other sources of variation (namely, tree apparency and neighbour diversity and identity) were controlled for, anti-herbivore defences had no significant effect on herbivory. Because neighbour identity and diversity partially modified leaf chemistry, this result suggests that by itself, leaf chemistry only explains a small amount of variation in herbivory and that other unmeasured traits (e.g. volatile organic compounds, induced defences, Zakir et al., 2013) or other processes independent of leaf traits (e.g. top-down control by predators, Esquivel-Gómez, Abdala-Roberts, Pinkus-Rendón, & Parra-Tabla, 2017; Moreira, Mooney, et al., 2012; Muiruri, Rainio, & Koricheva, 2016) could account for the effect of neighbour diversity and identity on insect herbivory. This is in accordance with the results of a previous study using the same experimental design but focusing on oak (*Q. robur*, Castagneyrol et al., 2017) which showed that despite clear effects of tree neighbours on leaf traits, traits poorly explained variation in leaf insect herbivory (Castagneyrol et al., 2017).

There is growing evidence that below- and above-ground interactions among plants can alter the expression of direct and indirect (i.e. enemy-mediated) anti-herbivore defences and traits determining leaf nutritional quality (Glassmire et al., 2016; Kostenko et al., 2017; Moreira et al., 2014, 2017; Mraja et al., 2011; Nitschke et al., 2017; Walter et al., 2011). However, whether neighbour-induced changes in leaf chemistry mediates associational effects only received partial support in the literature. For instance, Moreira et al. (2014) reported that greater production of anti-herbivore chemical defences in mixtures of tree species and genotypes of mahogany (compared with monocultures) did not lead to concomitant patterns of insect herbivory. Likewise, Kostenko et al. (2017) reported that the effects of neighbours on arthropods associated to *Jacobaea vulgaris* were independent of changes in plant chemistry. Contrarily, neighbour diversity had indirect positive effects on herbivore diversity via reduced investment in anti-herbivore defences on pepper trees (Glassmire et al., 2016). By addressing the direct- and indirect-trait mediated

effects of neighbours, our results add to the growing body of knowledge on mechanisms controlling for insect damage on plants.

Insects caused more damage in stressful conditions, independent of leaf chemistry. Our results showed that defoliation increased in drought-stressed plants. These findings are partially in accordance with the plant stress hypothesis which predicts greater performance of foliage-feeding herbivores in stressed plants (Jactel et al., 2012; White, 1974). However, because irrigation had no effect on individual leaf traits (excepted starch content) or coordinates on PC axes, our results do not support the premise of the plant stress hypothesis that increased herbivory in stressed plants results from changes in leaf chemistry and in particular from the increased concentration of soluble sugars and secondary metabolites (Walter et al., 2011; White, 1974; Ximénez-Embún et al., 2016). We cannot exclude that other unmeasured traits such as concentration of free amino acids, induced secondary metabolites or emission of volatile organic compounds may have been the causal link between drought and insect herbivory. For instance, Gutbrodt, Dorn, and Mody (2011) reported that drought had no effect on induced defences in apple trees while constitutive defences were reduced in moderately stressed plants and increased in highly stressed plants. Because we only targeted undamaged leaves for trait analyses, we are not able to tease apart the response of constitutive vs. induced defences.

Associational effects were contingent upon water stress. Leaf-chewing damage and leaf-miners incidence in early season were on average higher in mixtures than in monocultures, but only under stressful conditions. Importantly, this pattern remained unaltered when leaf traits were included in the statistical model. It is important to note that we were able to detect significant effects of the interaction between tree species diversity and water treatments on herbivory with our rather small sample size ( $N = 96$ ), suggesting that these effects were strong.

The state of ORPHEE experiment at the time we performed this study was such that birch concentration, frequency and apparency (sensu Castagneyrol et al., 2017) were comparable between irrigated and non-irrigated plots, which makes it unlikely that processes related to birch accessibility could explain the observed interaction between drought and tree diversity on herbivory. It is possible that drought increased species-specific differences in plant cues (e.g. emission of volatile organic compounds) used by herbivores to locate a suitable host within experimental plots (including trees and understory vegetation). This would result in stronger relative attractiveness of the fewer birches in non-irrigated mixtures as compared to monocultures (Hambäck et al., 2014).

Because the irrigation treatment was initiated only 1 year before our measurements, a critical gap of this study would be that we cannot address temporal dynamics in the associational effects. In particular, and although the irrigation treatment affects insect herbivory (this study), avian predation (Castagneyrol et al., 2017) and understory vegetation (B. Castagneyrol, unpubl. data), it may take longer to have detectable effects on tree height and apparency. Here, we show that more apparent birches had leaves with lower quality (lower water content, higher levels of anti-herbivore defences, Figure S5). Yet, given the known trade-offs between growth and defences (Herms & Mattson,

1992), it is possible that the relationship between defence traits and apparency will be affected by irrigation treatments in the long term.

Finally, it is important to note that we did not control water stress directly; the irrigation treatment aimed at alleviating water stress caused by summer drought. Hence, the difference in water stress intensity between irrigated and non-irrigated plots is therefore highly dependent on climatic conditions. Yet, summer 2016 was one of the driest over the last 60 years and we provided several lines of evidence that the experimental set up created two contrasted situations in terms of water availability. Importantly, drought alleviation was independent of tree species composition and affected not only birch but also oaks and pines planted in the ORPHEE experiment (Castagnéyrol, unpublished data). Thus, we are confident that our results did show the interactive effects of water stress and neighbours on leaf traits and insect herbivory. However, there is growing evidence that the effects of water stress on herbivory are more complex than that initially suggested by the plant stress hypothesis. In particular, stress frequency (Banfield-Zanin & Leather, 2014; Huberty & Denno, 2004) and stress intensity (Mody, Eichenberger, & Dorn, 2009; Sconiers & Eubanks, 2017) emerged as key drivers of insect response. It will be possible to test these effects with our experiment in the future by comparing the relative strength of diversity and irrigation effects on insect herbivory across years varying in the amount of summer rainfalls.

## 5 | CONCLUSIONS AND PERSPECTIVES

A long held view in ecology holds that insect herbivory on a given plant depends on the diversity and identity of its neighbours (Atsatt & O'dowd, 1976). However, despite dozens of empirical and experimental studies reporting evidence for associational resistance and susceptibility (reviewed by Barbosa et al., 2009; Moreira et al., 2016), predicting the strength and direction of associational effects remains challenging. Here, by demonstrating that associational effects are contingent upon abiotic constraints, we bring another degree of complexity into our understanding of the mechanisms controlling for insect herbivory among different neighbours.

Altogether, our results suggest that neighbours have indirect trait-mediated effects on insect herbivory, but neighbours' effects differentially affect two key components of leaf chemistry, namely leaf nutritional quality and anti-herbivore defences. Importantly, trait-mediated effects only partially accounted for the variability in insect herbivory on trees among different neighbours suggesting that unmeasured factors other than changes in leaf quality or host availability (i.e. concentration, frequency) and apparency may have contributed to explain the effect of neighbour diversity and identity on leaf herbivory.

While previous studies on birch reported greater resistance to insects in mixed forest (Muiruri, Milligan, Morath, & Koricheva, 2015; Setiawan, Vanhellefont, Baeten, Dillen, & Verheyen, 2014; Vehviläinen, Koricheva, Ruohomäki, Johansson, & Valkonen, 2006), we found the opposite pattern. Yet, the ORPHEE experiment is located at the South-West margin of *B. pendula* geographical range where it is more likely to suffer from summer heat and drought

(Atkinson, 1992). Together with recent studies (Haase et al., 2015; Kambach et al., 2016), our results challenge the view that associational resistance to insect herbivores is a pervasive phenomenon in forest ecosystems. At the contrary, they warn that abiotic stresses (e.g. drought, temperature, Kambach et al., 2016; Ratcliffe et al., 2017) are likely modifiers of associational effects that must be accounted for to better anticipate the future of plant-plant-insect interactions in the face of climate change.

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## AUTHORS' CONTRIBUTIONS

B.C. and H.J. conceived the experiment; B.C. and X.M. acquired the data; B.C. analysed the data and wrote the first draft which was commented, edited and improved by H.J. and X.M.

## DATA ACCESSIBILITY

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.73md8h4> (Castagnéyrol, 2018).

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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