Eco-evolutionary outsiders: Establishing in a distantly related neighbourhood delays and reorganizes nutrient recycling

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Abstract

Rapid environmental change forces long-lived plants like trees to immigrate into zones still occupied by phylogenetically distantly related species. Does such phylogenetic isolation (PI) change the trees' ecosystem functioning such as litter decomposition? We studied oaks (*Quercus petraea*) of low and high PI, reciprocally transplanting their litters to identify effect of aboveground litter quality and belowground decomposer biota. Across 8 and 14 months we quantified decomposition (mass loss, C-loss and N-loss), decomposer biota (Acari, Collembola, microbes) and $^{13}C/^{12}C$ ratio. Across 14 months, aboveground PI retarded decomposition (mass and C loss). Across 8 and 14 months, above- and belowground PI extensively altered relationships between decomposition and abundances/diversities of different soil biota, reduced microbial activity and $^{13}C/^{12}C$ ratios. Overall, coexistence of trees with distant relatives impedes and severely re-organizes C and N recycling. Such negative ecosystem feedback might prevent trees from tracking and conserving abiotic niches under environmental change.

Introduction

There is a tendency of species to retain the ancestral niche of their lineage, i.e. niche conservatism, and different niches may hence be occupied by different lineages (Crisp & Cook 2012). Niche conservatism is the necessary condition for related species to coexist locally (Prinzing *et al* . 2016, 2017). However, if environmental conditions change rapidly and organisms are sessile and long lived then niche conservatism may produce local neighbourhoods that are distantly related, such as a tree immigrating into a zone that recently became abiotically suitable but is still occupied by distant relatives. This and other processes result in phylogenetic isolation between a focal organism and its neighbours (Cavender-Bares *et al* . 2009). Coexistence with distant relatives may change species interactions to the advantage or the disadvantage of the focal individual (Webb *et al* . 2002; Yguel *et al* . 2011; Verdú *et al* . 2012; Gerhold*et al* . 2015; Prinzing *et al* . 2017). However, it remains unknown what are the consequences of coexistence with distant relatives for the plant's contribution to ecosystem functioning, such as the recycling of its own carbon (C) and nitrogen (N) through decomposition. For sessile, big, long lived organisms like trees that grow and germinate in their own litter such an ecosystem feedback would be essential for maintenance and reproduction - and thereby the capacity of species to track conserved niches under rapid environmental change (Ackerly 2003).

Plant litter decomposition is an important and complex ecosystem function depending on multiple abiotic

(*e.g.* temperature and moisture conditions), biotic factors (*i.e.* plants and soil organisms) and their interactions (Coûteaux *et al* . 1995; García-Palacios *et al* . 2013; Santonja *et al* . 2015). This process is governed, firstly, by the quantity and quality (*i.e.*physico-chemical characteristics) of litter that was produced by plants, providing microhabitats and food resources for soil organisms. These soil organisms are then the main actors of the decomposition process controlled by i) the abundance and diversity of soil organisms (Fig. 1a, Trajectory T1, Cornwell & Weedon 2014) and ii) the efficiency by which these soil organisms decompose a given litter (Fig. 1a, Trajectory T2). Even though the process is largely driven by soil organisms, a part of the decomposition process is abiotically driven resulting from leaching losses of mineral ions and small organic molecules (Fig. 1a, Trajectory T3). These different trajectories will leave different statistical signatures (Table 1). Below, we will hypothesize how phylogenetic isolation of a focal tree might operate on each of these trajectories.

We hypothesize that phylogenetic isolation of a focal tree from its neighbors influences the decomposition of the tree's litter due to aboveground mechanisms, i.e. mechanisms changing litter quality (Fig. 1b). Specifically, phylogenetic isolation aboveground (PIA) strongly reduces phytophagy of the focal tree (Yguel *et al* . 2011) and phytophagy is closely linked to the leaf litter quality (Schädler*et al* . 2003). For example, phytophagy can induce the production of secondary metabolites such as phenolics (Schultz & Baldwin 1982; Karban & Myers 1989; Coley & Barone 1996; Strauss & Agrawal 1999). These compounds negatively affect abundance and efficiency of decomposer organisms but also the abiotic decomposition of litter (Trajectories T1 and T2 in hypothesis PIA1, and PIA2 in Table 1). On the other hand, phytophagy can increase the leaf damage and in turn increase the accessibility of leaf litter for soil decomposers (PIA3, Cárdenas & Dangles 2012), as well as for moisture and desiccation (PIA4, Ritchie*et al* . 1998). Finally, phylogenetic isolation from neighbours may also impose a stress on the focal trees due to unfavorable competitive or microclimatic conditions (*e.g.* lower soil pH or moisture conditions). Such stress may trigger increased investment into, again, phenolics and/or carbon-rich compounds (Fernandez *et al* . 2016), impacting decomposers numbers and efficiency as well as abiotic decomposition of litter (PIA5 and PIA6).

We also hypothesize that phylogenetic isolation of a focal tree from its neighbors influences decomposition of the tree's litter due to belowground mechanisms (Fig. 1b). On the one hand, a phylogenetically distant neighborhood produces a litter that is dominated by distantly related plant lineages and may select for soil biota particularly capable of decomposing this dominant litter (Ayres *et al* . 2009; Austin *et al* . 2014; Pan *et al* . 2015; Cheng & Yu 2020), but naïve for decomposing the litter of the focal tree. Decomposition of the focal tree's litter might hence suffer from a phylogenetic away-field disadvantage (PIB1 in Table 1), a so far untested phenomenon equivalent to taxonomic away-field disadvantage that has been often demonstrated (Gholz *et al* . 2000; Negrete-Yankelevich *et al* . 2008; Vivanco & Austin 2008). On the other hand, mixing of litter from distantly related focal and neighbor trees may trigger complementarity effects: transfer of nutrients among litters might increase decomposer abundance, diversity and activity and hence decomposition (PIB2, Lummer *et al* . 2012; Handa *et al* . 2014; Porre *et al* . 2020). Transfer of toxins among litters, in contrast, might decrease decomposers and their efficiency and hence decomposition (PIB3, Hättenschwiler & Vitousek 2000; Gessner *et al* . 2010). Finally, some neighboring lineages may degrade the physical environment in which the focal tree decomposes, *e.g* . by decreasing soil pH (PIB4) and soil moisture conditions (PIB5) or by increasing thermal fluctuations due to delayed budburst (PIB7, Yguel*et al* . 2014) and thereby a shorter vegetation period (PIB8). Other neighboring lineages might improve the physical environment in which the focal tree decomposes, *e.g.* by decreasing soil soaking and hence increasing aeration (PIB6, Cornelissen *et al* . 2017; Dias *et al* . 2017). Overall, phylogenetic isolation of a focal tree affects the pool of decomposers, their resources and the physical background, but consequences for decomposition remain unknown.

As decomposition below a given tree will be the result of a mixture of both aboveground and belowground processes, we designed a litter bag experiment for trees growing among phylogenetically closely or distantly related neighbors, *i.e.* under low or high phylogenetic isolation (PI, Fig. 1c). To our knowledge, this is the first 'phylogenetic litter-transplantation' experiment. Such an approach exposes litter from both high-PI and low-PI trees under a given tree to identify effects of PI operating *via* aboveground processes. Moreover, the approach exposes litter from a given tree under both high-PI and low-PI trees to identify the effects of

PI operating *via* belowground processes. We studied oaks (*Quercus petraea*), a system for which effects of phylogenetic isolation on enemy pressure and budburst have been demonstrated already (*e.g.* Yguel *et al* . 2011; Yguel*et al* . 2014) and for which major shifts in ranges of suitable climate are predicted (Hansen *et al* . 2001; Iverson & Prasad 2001; Barton 2002).

To identify ecosystem consequences of coexistence with distantly related neighbours we tested a set of increasingly complex predictions from hypotheses in Table 1: (1) Phylogenetic isolation above (PIA) and/or belowground (PIB) per se changes the rate at which litter decomposes (in terms of mass loss, C-loss and N-loss). (2) PIA and/or PIB changes the trajectories by which litter decomposes: the abundances and diversities of different soil biota controlling decomposition, the efficiency by which a given group of soil biota at a given abundance decompose litter, the putatively abiotic effects that are not attributable to soil organisms considered (Fig. 1a). As soil biota we accounted for the dominant groups Acari, Collembola and microorganisms, and, for a subsample, for fungi. Moreover, trajectories invoking higher decomposer activities should lead to a strong relative accumulation of^{13}C (Bowling *et al* . 2008) and we hence tested whether PIA and/or PIB changes the^{13}C/^{12}C signatures. (3) The effects of PIA or PIB can be replaced, and hence likely explained by, litter traits and/or environmental characteristics as outlined above and in Table 1.

Materials and Methods

Site description and phylogenetic isolation estimation

Our study was conducted in the Forest of Rennes (surface area: 2000 ha), Brittany (France). The forest and selection of trees was detailed in Yguel *et al* . 2011 (Supplementary Methodology S1). Overall 11 pair of oaks with contrasting phylogenetic distance of neighbours were chosen, with neighbours belonging to 10 different species. For each focal oak, phylogenetic isolation was quantified as its mean phylogenetic distance to all neighboring trees with which its crown was in contact, and phylogenetic distances were extracted from published phylogenies following procedures applied previously (Vialatte*et al* . 2010; Yguel *et al* . 2011; Supplementary Methodology S1).

Experimental design studying decomposition of oak litter

For each pair of the focal oaks, we sampled the litter at leaf fall by gently shaking the branches and collecting the leaves before falling on the ground to avoid contamination by soil microbes and arthropods prior to the experimental treatment. Oak litter was air dried in the lab at ambient temperature for at least 2 weeks. For each tree, four samples of each oak litter (about 10 g in equivalent dry weight) were placed in litter bags (25 cm × 15 cm) with 5 mm-mesh size allowing colonization by microbes and soil fauna (Santonja *et al* . 2017). Moreover, for each tree, five samples were weighted and oven-dried to estimate the water content of the initial litter, *i.e.* (oven dry weight-air dry weight)/air dry weight. This ratio permitted to estimate the oven-dry weight equivalents of litter samples in the litter bags. Within each pair of trees, litters from the high-phylogenetic isolation tree were exposed below that tree and below the tree of low phylogenetic isolation, and inversely for the low-phylogenetic isolation tree (Fig. 1c). Therefore, a given litter bag was characterized by phylogenetic isolation of the oak from which the litter was sampled and the oak where the litter was incubated, respectively phylogenetic isolation above- and belowground PIA and PIB. Litter bags were posed at approx. 1 m from the trunk of the focal tree, i.e. close enough to avoid impact from other trees. Additional metal-mesh protection was used to avoid physical disturbance by large mammals (wild boars, humans). We harvested the litter bags twice, after 8 months and after 14 months. This gives 8 litter bags for each pair of oaks and a total of 88 litter bags.

Measurements of microbial biomass, arthropods, and fungal community composition

Litter bags were retrieved and transported in individual plastic bags to the laboratory. For each litter bag, a small subsample of the remaining litter was taken and attached mineral soil was brushed off. Microbial biomass of that small subsample was analyzed using the substrate-induced respiration (SIR) method (Anderson *et al* . 1978). The microbial respiratory response was measured in an electrolytic O_2microcompensation apparatus at hourly intervals for 24 h at 22 °C (Scheu 1992). Microbial biomass was measured after the

addition of glucose to saturate the catabolic activity of microorganisms. The maximum initial respiratory response (MIRR: ml O_2 g^{-1}DW h^{-1}) was calculated as the mean of the lowest three readings within the first 10 h and the microbial biomass for each litter bag was calculated as $C_{mic} = 38 \times$ MIRR (mg C_{mic} g^{-1} DW; Beck*et al* . 1997). After measuring microbial biomass, soil arthropods were extracted from the entire remaining litter by heat and stored in saturated salt solution (NaCl) at -10 °C for further measurement and identification (Pausch *et al* . 2016). Soil arthropods were counted and identified to class level if possible by light microscopy. The vast majority of arthropods were Acari or Collembola. Fungal community composition in the remaining litter after 8 months were measured using ITS1-ITS2 barcoding primers to sequence the total fungal community (Supplementary Methodology S2), and we calculated the total abundance and Simpson diversity index for each litter bag (Rosenzweig 1995).

Measurements of litter decomposition and litter traits

After the extraction of soil arthropods, litter was oven dried at 65 °C to weight constancy and weighed at 0.1 g. The same was done with litter that had not been exposed. The mass loss was then calculated as (pre-exposure – post-exposure)/ pre-exposure. Oven-dried litter was grinded using a ball mill. Carbon and Nitrogen concentrations were determined by using an elemental analyser (N1500, Carlo Erba, Milan, Italy). The changes in C and N concentrations were calculated in the same way as for mass loss. A decrease of C concentration reflects rapid decomposition. Inversely, an increase of N concentration reflects an accumulation of N in the litter due to decomposition, improving resource conditions for plants. We refrained from using changes of absolute C or N values to avoid bias from among-site variation of C or N. For instance, high-N sites would always show higher absolute changes of N than low-N sites. Subsequently, leaf carbon isotope was analyzed using an isotope ratio mass spectrometer (Isoprime100; Isoprime Ltd, UK) and the absolute atom percentage of carbon isotope was used for further analysis. Total phenolic concentration of initial litter was measured colorimetrically using the method of Santonja *et al* . (2015) with gallic acid as a standard. Leaf powder samples (250 mg) were suspended in 20 mL of a 70% aqueous methanol, shaken for 1 h and then filtered (0.45 µm filter). Filtered extracts (0.25 mL) were mixed with 4 mL of distilled water, 0.5 mL of saturated aqueous Na_2CO_3 and 0.25 mL of Folin-Ciocalteu reagent. After 60 min, phenolic concentrations were determined at 765 nm on the same UV/Vis spectrophotometer (Biomate 3, Thermo Electron Corporation®) and expressed as mg of gallic acid equivalent. g^{-1} DW.

Statistical analysis

In all analyses, we identified and excluded outliers as explained in Supplementary Methodology S3. We used multiple regression analyses to test how phylogenetic isolation aboveground (PIA) and belowground (PIB) conjointly affect i) the decomposition parameters (litter mass loss, C loss, N loss and ^{13}C isotope ratios) and ii) the decomposer biota, *i.e.* Acari abundance, Collembola abundance, and microbial biomass after 8- and 14-month decomposition, and fungal abundance and fungal diversity after 8 month decomposition. The regression results were illustrated in partial residual plots.

We then explored the role of different trajectories by which PIA or PIB may affect decomposition (Table 1): (i) by changing the abundance or diversity of a given group of decomposers, *i.e.* PIA or PIB influences significantly a particular group of decomposers (abundance or diversity) which in term influences decomposition (either mass loss or C loss or N loss either at 8 or at 14 months); (ii) by changing the efficiency of a given decomposer group (abundance or diversity),*i.e.* a significant interaction term between either of the phylogenetic isolations and the decomposer group in their effect on decomposition; or (iii) by affecting decomposition *via* abiotic conditions, *i.e.* PIA or PIB maintain a significant effect on decomposition after accounting for abundances and diversities of decomposer groups and the corresponding interaction terms (assuming that all pertinent decomposer groups have been accounted for). Testing the influence of PIA and PIB on decomposers as part of relationship (i) was explained in the before paragraph. The remaining relationships are explained below.

We explained decomposition by including PIA and PIB and decomposer biota as predictors, as well as the interaction terms of decomposers with both PIA and PIB. Due to the large number of decomposer biota

as predictors we took a stepwise approach to select the most pertinent interaction terms with decomposer variables. For each of the six decomposition variables to be explained, we first carried out five distinct statistical models corresponding to five biotic predictors: Acari abundance, Collembola abundance, and microbial biomass. Each model tested the effects of PIA, PIB, one biotic predictor and its interactions with PIA and PIB. Finally, in order to decipher the relative contributions of the five biotic predictors to explain litter decomposition, we created a last model in which we included all the significant predictors of the five previous models. The full models were then simplified to determine the most parsimonious models using the 'stepAIC' function of the 'BMASS' package, an established model selection procedure with both forward and backward selection algorithms, which ranks all candidate models (all initial predictor variables included in the full model) based on the lowest AICs (Crawley 2013). We conducted a separate analysis for fungal abundances and diversities, as these were only available for the 8-month sampling. The procedure of including interaction terms was the same as before.

In the end, we further explored whether the observed direct or interaction effects of PIA or PIB could be explained by litter traits such as leaf phenolics, leaf phytophagy or leaf litter C/N ratios, or by characteristics of the abiotic environment, as suggested by the mechanisms hypothesized in the Introduction and in Table 1. We did so by replacing either PIA or PIB by a given trait or environmental characteristic (as listed in Supplementary Table S1) and repeated the procedure for different traits/environmental characteristics. Statistical analyses were performed with the R software (version 3.3.3).

Results

Effects of phylogenetic isolation aboveground (PIA) and belowground (PIB) on litter decomposition, soil biota and carbon isotope

Loss of mass, C and N . PIA increased C loss after 8 months, decreased C loss after 14 months and decreased mass loss after 14 months (Fig. 2). PIB decreased litter C loss after 8 months (Fig. 2). PIA and PIB did not affect mass loss or N loss after 8 months.

Abundances of decomposers . PIA decreased microbial biomass after 14 months (Fig. 2). PIB decreased microbial biomass and Collembola abundance after 8 and 14 months, and increased Acari abundance after 8 months (Fig. 2). PIA or PIB did not affect fungal abundance or diversity after 8 months (Supplementary Figure S1).

Carbon isotope . PIA decreased litter$^{13}C/^{12}C$ after 8 months, while PIB decreased litter $^{13}C/^{12}C$ after 14 months of decomposition (Fig. 3).

Combined effects of PIA, PIB and soil biota on litter decomposition

We here focus on the main and interaction effects of PIA and PIB and on the main effects of those decomposers that were in turn significantly affected by PIA and PIB as outlined in the last chapter. We interpret significant main effects together with significant interaction terms of the same variable as main effects were no less important than interaction terms.

Analyses accounting for Collembola, Acari and microbial biomass at 8 and 14 months

Mass loss . PIA did not directly affect mass loss after 8 and 14 months. PIB increased mass loss after 8 months, but decreased mass loss after 14 months (main effects in Table 2). The observed positive effects of PIB after 8 months were replaceable by shorter vegetation period, delayed budburst and decreasing soil moisture (Supplementary Table S2). Moreover, Acari abundance (which was increased by PIB after 8 months, Fig. 2) directly increased mass loss after 8 and 14 months (main effects in Table 2). There was a negative interaction between PIB and Acari abundance on mass loss after 8 months, in which PIB was replaceable by delayed budburst (Table 2; Supplementary Table S2). There was a negative interaction between PIB and Collembola abundance, in which PIB was replaceable by longer vegetation period. In addition, there was a positive interaction between PIB and microbial biomass on mass loss after 14 months, in which PIB was replaceable by increasing soil moisture (Table 2; Supplementary Table S2).

Carbon loss . PIA directly increased C loss after 8 months, but PIB directly decreased C loss after 8 months (main effects in Table 2). The observed positive effect of PIA on C loss was replaceable by decreasing phytophagy, and the observed negative effects of PIB was replaceable by delayed budburst (Table 2; Supplementary Table S2). Moreover, Collembola abundance (which was decreased by PIB, Fig. 2) directly increased C loss after 14 months. There was a negative interaction between PIA and Collembola abundance after 14 months, in which PIA was replaceable by both increasing phytophagy and decreasing phenolics (Supplementary Table S2). Acari abundance (which was decreased by PIB after 8 months) directly decreased C loss after 8 and 14 months. There was a positive interaction between PIB and Acari abundance, in which PIB was replaceable by increasing budburst or decreasing soil pH (Table 2; Supplementary Table S2).

Nitrogen loss . Only PIB directly decreased N loss after 8 months (main effects in Table 2). This negative effect of PIB on N loss was replaceable by longer vegetation period (Supplementary Table S2). Moreover, microbial biomass (which was decreased by PIB after 8 months; Fig. 2) directly decreased N loss after 8 months. No interaction effects were found between either PIA or PIB and soil biota (Table 2).

Analyses accounting for Fungal abundance and diversity at 8 months

Mass loss . PIA directly increased mass loss, but the effect was much weaker than interaction term and hence not interpretable, and there was a negative interaction between PIA and Fungal diversity on mass loss (Supplementary Table S3).

Carbon loss . PIA tended to directly increase C loss, and there was a negative interaction between PIB and Fungal diversity on C loss (Supplementary Table S3).

Nitrogen loss . PIA directly decreased N loss, and there was a tendency of a positive interaction between PIA and Fungal diversity on N loss (Supplementary Table S3).

Discussion

Our study showed phylogenetic isolation (PI) of a focal oak might significantly affect and mostly reduce the decomposition of its own litter. Phylogenetic isolation operates *via* both aboveground (PIA) and belowground (PIB): aboveground effects often being replaceable by low phytophagy, and belowground effects by microclimate-drivers such as tree vegetation period, budburst or decreasing soil moisture. These patterns are consistent with predictions from different mechanisms presented in Table 1. Accounting for multiple trajectories by including *decomposer*PI* interaction terms was important and permitted to identify significant direct or indirect effects of PI on decomposition in 7 out of 9 analyses on 5 out of the 6 dependent variables, compared to 3 out of 6 models that did not account for multiple trajectories. The biotic trajectories T1 and T2 might be important aboveground, representing 3 out of 6 observed relationships of decomposition to PIA, mainly involving fungal diversities and later Collembola abundance. The biotic trajectories T1 and T2 are even dominant belowground, representing 5 out of 6 relationships of decomposition to PIB, involving first Collembola and Acari abundance, but later microbial biomass. Moreover, we find a decrease in ^{13}C, first with PIA, then with PIB, suggesting that PI reduces the role of biotic trajectories of decomposition besides decreasing overall rates of decomposition.

We experimentally separated effects of above- and belowground PI, but did not manipulate PI as such, nor the presence of particular decomposer species or particular abiotic conditions. Manipulating PI would be impossible given the lifespan of trees. Replication and blocking - PI and non-PI trees at less than 150 m - limit the problem of correlational evidence, but do not entirely resolve it. Also, manipulating particular decomposer species is difficult, but would be needed to assess whether a significantly positive *PI * decomposer* interaction reflects rather a shift towards more efficient decomposer species, a shift within each decomposer species towards increased decomposition performance, or a support of decomposers by nutrients from dung and urine of the herbivores above (Cherif & Loreau 2013). Finally, manipulating abiotic conditions might be more doable and would represent a direct prove for abiotically-mediated effects of PI, *i.e.* our trajectory T3. Overall, we profit from a quasi-experiment of PI established by foresters, and independently manipulate above- and belowground. However, we only use correlational approaches to explain observed effects of PI

by different mechanistic pathways. Hence, conclusion about mechanisms remain interpretations of patterns, causal terminology refers to statistical relationships rather than direct proof of mechanisms.

For simplicity we will from now focus on explaining the relationships of PIA and PIB alone on decomposition in analyses only accounting for combined effects of PIA and PIB (Fig. 2). To do so, we will use trajectory analyses (Fig. 1) and the replacement of variables, verifying the predictions from Table 1.

Relationships of decomposition to phylogenetic isolation belowground, PIB

PIB mainly related to the lower C loss at 8 months in analyses only accounting for PIA and PIB (Fig. 2). This relationship was partly direct, *i.e.* the abiotic trajectory T3, and was replaceable by tree phenological effects on microclimate, as predicted by hypotheses PIA7 and PIA8. Partly this relationship appeared to be mediated by decomposers (trajectories T1 and T2). First, PIB increased Acari abundance after 8 months, which in turn related to strongly decreased C loss (albeit the PIB*Acari interaction had the inverse effect). Second, PIB rendered the effect of fungal diversity on C loss more negative. These relationships are partly consistent with predictions of PIB1 and PIB3, *i.e.* a naiveté of decomposers in or transfer of toxins from the ambient phylogenetically distant litter (Table 1, consistent with decomposability of functionally uniform litter; Grossmann *et al* . 2020). At 14 months PIB did not relate to C loss (in analyses only accounting for PIA and PIB). Nevertheless, at 14 months PIB decreased Collembola abundance which in turn related positively to C loss. Possibly, this potential negative Collembola-mediated effect of PIB contributed to the negative effect of PIB on ^{13}C after 14 months. Overall, there appears to be a temporal phylogenetic home-field advantage where C loss is stronger in a "home litter" produced by closely related neighbourhood (Aponte *et al* . 2012). As a consequence, oaks may have difficulties to penetrate into a phylogenetically distant neighbourhood.

Regarding mass loss and N loss, litter mostly decomposed equally well when exposed in a phylogenetically closely or distantly related belowground neighbourhood. Processes that decrease decomposition were likely compensated by such that increase decomposition, and we could indeed identify the corresponding opposite relationships when comparing different trajectories. Specifically, PIB directly increased mass loss and decreased N loss after 8 months, while decomposer-mediated effects were opposite: PIB decreased the effect of Collembola or Acari on mass loss, and PIB decreased microbial biomass itself decreasing N loss. Moreover, PIB might also have relatively little effect on mass loss, as in our system high PIB often corresponds to a gymnosperm-dominated neighbourhood with highly recalcitrant litter (Kaneko & Salamanca 1999; Cornwell *et al* . 2008; Berendse & Scheffer 2009). Being "trained" in decomposing such recalcitrant litter, decomposers in a high PIB environment might efficiently reduce the mass of any litter, with little preference for their "home" litter (Milcu & Manning 2011; Wallenstein *et al* . 2013). Finally, mass loss might be maintained in a high PIB environment by increased enzymatic activities of ectomycorrhizal fungi (Yguel *et al* . 2014; Martin Schädler & Daniel J. Ballhorn 2016). Overall, despite constancy in mass loss and N loss among low and high PIB neighbourhoods, the trajectories of decomposition have become different, and there is a "home-field functioning" (Vivanco & Austin 2008; Ayres *et al* . 2009). Shift in belowground trajectories of functioning while maintaining overall rate of functioning suggests redundancy among different trajectories of decomposition, and a flexibility to shift in neighbourhood. Nevertheless, different trajectories may depend on different litter traits and penetration into a phylogenetically distantly related neighbourhood might impose new selection pressures on litter traits (Guénon *et al* . 2017).

Relationships of decomposition to phylogenetic isolation aboveground, PIA

First, PIA shows no effect on mass loss after 8 months, and a negative effect after 14 months (in analyses only accounting for PIA and PIB, Fig. 2). Analysis of trajectories fails to reproduce this negative effect and yields among others a negative effect of PIA on the role of fungal diversity on mass loss after 8 months. Possibly, the negative effect of PIA on ^{13}C after 8 months suggests shortage of easily decomposable compounds (Benner *et al* . 1987), reducing the mass loss at 14 months (consistent with hypothesis PIA5). Second, PIA shows a positive effect on C loss after 8 months (in analyses only accounting for PIA and PIB, Fig. 2). This effect is explicable by a direct, abiotic effect of PIA (trajectory T3), which in turn, is replaceable by low herbivory

(hypothesis PIA2): less attacked leaves might have kept more labile and soluble carbon rendering these leaves abiotically more degradable. In contrast, PIA shows a negative effect on C loss after 14 months. Analyses of trajectories suggests that this relationship is mediated by a decline in the relationship between Collembola and C loss, the latter being replaceable and possibly explicable by reduced phytophagy. These results are consistent with hypothesis PIA3: low phytophagy damage might reduce the access to leaves for decomposers (Ritchie *et al* . 1998). Finally, PIA shows no overall effect on N loss, despite a statistical marginally significant, positive effect on the role of fungal abundance for N loss after 8 months. This interaction effect is replaceable by high litter phenolics consistent with hypothesis PIA5, i.e. an increased competition for resources and/or allelopathic interactions between oaks and distantly related gymnosperms increasing phenolics and decreasing litter quality for decomposers (Fernandez *et al* . 2016).

Overall, litter that is produced in a neighbourhood of closely related lineages becomes more decomposable in the long run. This can be considered as a form of a phylogenetic home-field advantage, albeit one that is non-standard for two reasons. First this positive effect of neighbourhood results from aboveground processes, which so far have not been accounted for in the home-field research on decomposition (Ayres*et al* . 2009; Freschet *et al* . 2012; Veen *et al* . 2015). Second, home-field advantage among plants as proposed in the paleontological literature (DiMichele & Bateman 1996), has been explained by competitive superiority "at home". In the present case, however, evidence suggests that home-field advantage can be explained by the increased support from decomposers (see also Bardgett & van der Putten 2014).

Conclusion

Trees may find themselves back in a distantly related neighbourhood if niches were conserved in the past and at present (i) environments change rapidly, requiring colonization of sites still occupied by distantly related trees, or (ii) environments remain constant and individuals leave their ancestral niche and converge with niches of distantly related species. Such coexistence of trees with distant relatives appears to impede and severely re-organize carbon and nitrogen recycling. Processes involved operate through above- and belowground mechanisms, appear to involve abiotic drivers of decomposition in some cases, and biotic drivers in many others, notably fungi for aboveground effects and Acari and Collembola for belowground effects of phylogenetic isolation. Such mechanisms driven by local phylogenetic isolation might ultimately contribute to feedbacks between niche-evolution and ecosystem functioning (Srivastava *et al* . 2012; Prinzing *et al* . 2017): they might prevent trees from tracking and conserving niches under environmental change.

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References

Ackerly, D.D. (2003). Community assembly, niche conservatism, and adaptive evolution in changing environments. *Int. J. Plant Sci.* , 164, S165-S184.

Anderson, J. & Domsch, K. (1978). A physiological method for the quantitative measurement of microbial biomass in soils.*Soil Biol. Biochem.* , 10, 215-221.

Aponte, C., García, L.V. & Maranon, T. (2012). Tree species effect on litter decomposition and nutrient release in mediterranean oak forests changes over time. *Ecosystems* , 15, 1204-1218.

Austin, A.T., Vivanco, L., González-Arzac, A. & Perez, L.I. (2014). There's no place like home? An exploration of the mechanisms behind plant litter–decomposer affinity in terrestrial ecosystems. *New Phytol.* , 204, 307-314.

Ayres, E., Steltzer, H., Simmons, B.L., Simpson, R.T., Steinweg, J.M., Wallenstein, M.D. *et al.* (2009). Home-field advantage accelerates leaf litter decomposition in forests. *Soil Biol. Biochem.* , 41, 606-610.

Bardgett, R.D. & van der Putten, W.H. (2014). Belowground biodiversity and ecosystem functioning. *Nature* , 515, 505.

Barton, A.M. (2002). Intense wildfire in southeastern Arizona: transformation of a Madrean oak–pine forest to oak woodland.*Forest Ecol. Manag.* , 165, 205-212.

Beck, T., Joergensen, R., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H. *et al.* (1997). An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. *Soil Biology and Biochemistry* , 29, 1023-1032.

Berendse, F. & Scheffer, M. (2009). The angiosperm radiation revisited, an ecological explanation for Darwin's 'abominable mystery'. *Ecol. Lett.* , 12, 865-872.

Benner, R., Fogel, M. L., Sprague, E. K., & Hodson, R. E. (1987). Depletion of 13C in lignin and its implications for stable carbon isotope studies. Nature, 329(6141), 708–710.

Bowling, D. R., Pataki, D. E., & Randerson, J. T. (2008). Carbon isotopes in terrestrial ecosystem pools and CO2 fluxes. *New Phytol* ., 178(1), 24–40.

Cardenas, R. E., & Dangles, O. (2012). Do canopy herbivores mechanically facilitate subsequent litter decomposition in soil? A pilot study from a Neotropical cloud forest. *Ecol. Res.* , *27* , 975-981.

Cavender-Bares, J., Kozak, K.H., Fine, P.V. & Kembel, S.W. (2009). The merging of community ecology and phylogenetic biology. *Ecol. Lett.* , 12, 693-715.

Cheng, K., & Yu, S. (2020). Neighboring trees regulate the root-associated pathogenic fungi on the host plant in a subtropical forest. *Ecol. Evol.* , 10(9), 3932–3943.

Cherif, M. & Loreau, M. (2013). Plant–herbivore–decomposer stoichiometric mismatches and nutrient cycling in ecosystems.*Proc. Biol. Sci.* , 280, 20122453.

Coley, P.D. & Barone, J. (1996). Herbivory and plant defenses in tropical forests.*Annu. Rev. Ecol. Syst.* , 27, 305-335.

Cornelissen, J.H., Grootemaat, S., Verheijen, L.M., Cornwell, W.K., Bodegom, P.M., Wal, R. *et al.* (2017). Are litter decomposition and fire linked through plant species traits? *New Phytol.* , 216, 653-669.

Cornwell, W.K., Cornelissen, J.H., Amatangelo, K., Dorrepaal, E., Eviner, V.T., Godoy, O. *et al.* (2008). Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. *Ecol. Lett.* , 11, 1065-1071.

Cornwell, W.K. & Weedon, J.T. (2014). Decomposition trajectories of diverse litter types: a model selection analysis. *Methods Ecol. Evol.* , 5, 173-182.

Couteaux, M.-M., Bottner, P. & Berg, B. (1995). Litter decomposition, climate and liter quality. *Trends Ecol. Evol.* , 10, 63-66.

Crawley, M.J. (2013). The R book Second edition. John Wiley & Sons.

Crisp, M.D. & Cook, L.G. (2012). Phylogenetic niche conservatism: what are the underlying evolutionary and ecological causes? *New Phytol.* , 196, 681-694.

Dias, A.T.C., Cornelissen, J.H. & Berg, M.P. (2017). Litter for life: assessing the multifunctional legacy of plant traits. *J. Ecol.* , 105, 1163-1168.

DiMichele, W.A. & Bateman, R.M. (1996). Plant paleoecology and evolutionary inference: two examples from the Paleozoic.*Rev. Palaeobot. Palyno.* , 90, 223-247.

Fernandez, C., Monnier, Y., Santonja, M., Gallet, C., Weston, L.A., Prevosto, B. *et al.* (2016). The impact of competition and allelopathy on the trade-off between plant defense and growth in two contrasting tree species. *Front. Plant Sci.* , 7, 594.

Freschet, G.T., Aerts, R. & Cornelissen, J.H. (2012). Multiple mechanisms for trait effects on litter decomposition: moving beyond home-field advantage with a new hypothesis. *J. Ecol.* , 100, 619-630.

Garcia-Palacios, P., Maestre, F.T., Kattge, J. & Wall, D.H. (2013). Climate and litter quality differently modulate the effects of soil fauna on litter decomposition across biomes. *Ecol. Lett.* , 16, 1045-1053.

Gerhold, P., Cahill, J.F., Winter, M., Bartish, I.V. & Prinzing, A. (2015). Phylogenetic patterns are not proxies of community assembly mechanisms (they are far better). *Funct. Ecol.* , 29, 600-614.

Gessner, M.O., Swan, C.M., Dang, C.K., McKie, B.G., Bardgett, R.D., Wall, D.H. *et al.* (2010). Diversity meets decomposition. *Trends Ecol. Evol.* , 25, 372-380.

Gholz, H.L., Wedin, D.A., Smitherman, S.M., Harmon, M.E. & Parton, W.J. (2000). Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. *Glob. Change Biol.* , 6, 751-765.

Grossman, J. J., Cavender-Bares, J., & Hobbie, S. E. (2020). Functional diversity of leaf litter mixtures slows decomposition of labile but not recalcitrant carbon over two years. *Ecol. Monogr.* , ecm.1407–53.

Guenon, R., Day, T.A., Velazco-Ayuso, S. & Gros, R. (2017). Mixing of Aleppo pine and Holm oak litter increases biochemical diversity and alleviates N limitations of microbial activity. *Soil Biol. Biochem.* , 105, 216-226.

Handa, I. T., Aerts, R., Berendse, F., Berg, M. P., Bruder, A., Butenschoen, O., et al. (2014). Consequences of biodiversity loss for litter decomposition across biomes. Nature, 509, 218–221.

Hansen, A.J., Neilson, R.P., Dale, V.H., Flather, C.H., Iverson, L.R., Currie, D.J. *et al.* (2001). Global Change in Forests: Responses of Species, Communities, and Biomes: Interactions between climate change and land use are projected to cause large shifts in biodiversity. *AIBS Bulletin* , 51, 765-779.

Hattenschwiler, S. & Vitousek, P.M. (2000). The role of polyphenols in terrestrial ecosystem nutrient cycling. *Trends Ecol. Evol.* , 15, 238-243.

Iverson, L.R. & Prasad, A.M. (2001). Potential changes in tree species richness and forest community types following climate change. *Ecosystems* , 4, 186-199.

Kaneko, N. & Salamanca, E. (1999). Mixed leaf litter effects on decomposition rates and soil microarthropod communities in an oak–pine stand in Japan. *Ecol. Res.* , 14, 131-138.

Karban, R. & Myers, J.H. (1989). Induced plant responses to herbivory. *Annu. Rev. Ecol. Syst.* , 20, 331-348.

Lummer, D., Scheu, S. & Butenschoen, O. (2012). Connecting litter quality, microbial community and nitrogen transfer mechanisms in decomposing litter mixtures. *Oikos* , 121, 1649-1655.

Milcu, A. & Manning, P. (2011). All size classes of soil fauna and litter quality control the acceleration of litter decay in its home environment. *Oikos* , 120, 1366-1370.

Negrete-Yankelevich, S., Fragoso, C., Newton, A.C., Russell, G. & Heal, O.W. (2008). Species-specific characteristics of trees can determine the litter macroinvertebrate community and decomposition process below their canopies. *Plant Soil* , 307, 83-97.

Pan, X., Berg, M.P., Butenschoen, O., Murray, P.J., Bartish, I.V., Cornelissen, J.H. *et al.* (2015). Larger phylogenetic distances in litter mixtures: lower microbial biomass and higher C/N ratios but equal mass loss. *Proc. Biol. Sci.* , 282, 20150103.

Pausch, J., Kramer, S., Scharroba, A., Scheunemann, N., Butenschoen, O., Kandeler, E. *et al.* (2016). Small but active–pool size does not matter for carbon incorporation in below-ground food webs. *Funct. Ecol.* , 30, 479-489.

Porre, R. J., van der Werf, W., De Deyn, G. B., Stomph, T. J., & Hoffland, E. (2020). Is litter decomposition enhanced in species mixtures? A meta-analysis.*Soil Biol. Biochem.* , 145, 107791.

Prinzing, A., Powrie, L. W., Hennekens, S. M., Bartish, I. V., & Ozinga, W. A. (2016). "High-co-occurrence genera": weak but consistent relationships with global richness, niche partitioning, hybridization and decline. *Glob. Ecol. Biogeogr.* , 25, 55–64.

Prinzing, A., Ozinga, W.A., Brandle, M., Courty, P.E., Hennion, F., Labandeira, C. *et al.* (2017). Benefits from living together? Clades whose species use similar habitats may persist as a result of eco-evolutionary feedbacks. *New Phytol.* , 213, 66-82.

Ritchie, M.E., Tilman, D. & Knops, J.M. (1998). Herbivore effects on plant and nitrogen dynamics in oak savanna. *Ecology* , 79, 165-177.

Rosenzweig ML. (1995). Species diversity in space and time. New York, NY: Cambridge University Press.

Santonja, M., Fernandez, C., Gauquelin, T. & Baldy, V. (2015). Climate change effects on litter decomposition: intensive drought leads to a strong decrease of litter mixture interactions. *Plant Soil* , 393, 69-82.

Santonja, M., Rancon, A., Fromin, N., Baldy, V., Hattenschwiler, S., Fernandez, C. *et al.* (2017). Plant litter diversity increases microbial abundance, fungal diversity, and carbon and nitrogen cycling in a Mediterranean shrubland. *Soil Biol. Biochem.* , 111, 124-134.

Schadler, M., & Ballhorn, D. J. (2016). Beneficial Soil Microbiota as Mediators of the Plant Defensive Phenotype and Aboveground Plant-Herbivore Interactions. In U. Luttge, W. Beyschlag, B. Budel, & D. Francis, Resolving the Dryland Decomposition Conundrum: Some New Perspectives on Potential Drivers (Vol. 78, pp. 305–343). Cham: Springer International Publishing.

Schadler, M., Jung, G., Auge, H., & Brandl, R. (2003). Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos* , 103, 121–132.

Scheu, S. (1992). Automated measurement of the respiratory response of soil microcompartments: active microbial biomass in earthworm faeces.*Soil Biol. Biochem.* , 24, 1113-1118.

Schultz, J.C. & Baldwin, I.T. (1982). Oak leaf quality declines in response to defoliation by gypsy moth larvae. *Science* , 217, 149-151.

Srivastava, D.S., Cadotte, M.W., MacDonald, A.A.M., Marushia, R.G. & Mirotchnick, N. (2012). Phylogenetic diversity and the functioning of ecosystems. *Ecol. Lett.* , 15, 637-648.

Strauss, S.Y. & Agrawal, A.A. (1999). The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.* , 14, 179-185.

Veen, G., Freschet, G.T., Ordonez, A. & Wardle, D.A. (2015). Litter quality and environmental controls of home-field advantage effects on litter decomposition. *Oikos* , 124, 187-195.

Verdu, M., Gomez-Aparicio, L. & Valiente-Banuet, A. (2012). Phylogenetic relatedness as a tool in restoration ecology: a meta-analysis. *Proc. Biol. Sci.* , 279, 1761-1767.

Vialatte, A., Bailey, R.I., Vasseur, C., Matocq, A., Gossner, M.M., Everhart, D. *et al.* (2010). Phylogenetic isolation of host trees affects assembly of local Heteroptera communities. *Proc. Biol. Sci.* , 277, 2227-2236.

Vivanco, L. & Austin, A.T. (2008). Tree species identity alters forest litter decomposition through long-term plant and soil interactions in Patagonia, Argentina. *J. Ecol.* , 96, 727-736.

Wallenstein, M.D., Haddix, M.L., Ayres, E., Steltzer, H., Magrini-Bair, K.A. & Paul, E.A. (2013). Litter chemistry changes more rapidly when decomposed at home but converges during decomposition–transformation.*Soil Biol. Biochem.* , 57, 311-319.

Webb, C.O., Ackerly, D.D., McPeek, M.A. & Donoghue, M.J. (2002). Phylogenies and community ecology. *Annu. Rev. Ecol. Syst.* , 33, 475-505.

Yguel, B., Bailey, R., Tosh, N.D., Vialatte, A., Vasseur, C., Vitrac, X.*et al.* (2011). Phytophagy on phylogenetically isolated trees: why hosts should escape their relatives. *Ecol. Lett.* , 14, 1117-1124.

Yguel, B., Courty, P.-E., Jactel, H., Pan, X., Butenschoen, O., Murray, P.J. *et al.* (2014). Mycorrhizae support oaks growing in a phylogenetically distant neighbourhood. *Soil Biol. Biochem.* , 78, 204-212.

Tables

Table 1. Several mechanisms by which phylogenetic isolation (PI) of a focal tree might influence the decomposition of the tree's litter, and in *italics* the corresponding predictions. Mechanisms operate *via* either of three trajectories: mediated *via*decomposer abundances and diversities T1, decomposer efficiency T2, or non-biotic effects T3 (Fig. 1). See Introduction for explanations.

Hypothesis	Mechanism
Aboveground effects of PI	**Aboveground effects of PI**
Less phytophagy, hence less defense production against herbivores	Less phytophagy, hence less defense production against
PIA1	Litter less toxic for soil invertebrates
PIA2	Litter with more degradable C compounds
Less phytophagy, hence less damage on leaf litter	Less phytophagy, hence less damage on leaf litter
PIA3	Litter with less access for soil biota
PIA4	Slower moistening/desiccation
More stressful conditions, hence more defense production	More stressful conditions, hence more defense producti
PIA5	Litter more toxic for soil invertebrates
PIA6	Litter with less degradable C compounds
Belowground effects of PI	**Belowground effects of PI**
Ambient litter different from focal oak litter	Ambient litter different from focal oak litter
PIB1	Ambient decomposers naïve to focal oak litter
PIB2	Transfer of nutrients for decomposers
PIB3	Transfer of toxins for decomposers
PIB4	Ambient litter more acid
PIB5	Ambient litter with less water holding capacity
PIB6	Ambient litter more aerated
Altered vegetation period of the focal oak tree	Altered vegetation period of the focal oak tree
PIB7	Delayed budburst
PIB8	Shorter vegetation period

Table 2. Effects of phylogenetic isolation aboveground and belowground (PIA + PIB) on decomposition of oak litter, i.e. mass loss, carbon loss and nitrogen loss, based on multiple regression analyses, which involved the soil biota as predictive co-variables, as well as their interaction terms with PIA and PIB. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Residual degree of freedom ranged from 27 to 36. r-square and the adjusted r-square (adjr^2) for each model were listed in the table.

	Mass loss	Mass loss	Mass loss	Mass loss	Mass loss	Carbon loss	Carbon loss	C
	8 months	8 months		14 months	14 months	8 months	8 months	
	t-value	*P*-value		*t*-value	*P*-value	*t*-value	*P*-value	
PIA	1.76			1.05		2.37	*	
PIB	2.76	**		-2.39	*	-3.65	***	
Collembola abundance (CA)	2.87	**		0.57				

	Mass loss	Mass loss	Mass loss	Mass loss	Mass loss	Carbon loss	Carbon loss	C
Acari abundance (AA)	2.41	*		3.60	**	-3.13	**	
Microbial biomass (MB)	1.34			-1.25		-0.02		
PIA × CA								
PIA × AA								
PIA × MB				-1.29		-1.67		
PIB × CA	-2.20	*		-1.29				
PIB × AA	-2.40	*				2.59	*	
PIB × MB				2.51	*			
r^2	0.34	0.34		0.52	0.52	0.55	0.55	
Adjr^2	0.18	0.18		0.39	0.39	0.46	0.46	

Figure legend

Fig. 1. (a) Trajectories by which phylogenetic isolation (PI) of a focal tree might affect litter decomposition and how to, tentatively, infer them. Trajectory 1 (T1, single arrow line): effects of PI on decomposition mediated *via* the abundance and diversity of decomposers. Tentatively inferred from the abundance/diversity terms in multiple regression analyses [abundance (or diversity) + PI + abundance/diversity * PI -> Decomposition]. Trajectory 2 (T2, double arrow line): effects of PI on decomposition mediated*via* the efficiency of decomposers. Tentatively inferred from the abundance/diversity * PI interaction terms in multiple regression analyses [abundance (or diversity) + PI + abundance (or diversity) * PI -> Decomposition]. Trajectory 3 (T3, triple arrow line): effects of PI on decomposition mediated *via* the physical conditions of decomposition. Tentatively inferred from the PI terms in multiple regression analyses [abundance (or diversity) + PI + abundance (or diversity) * PI -> Decomposition]. Positive effects on decomposition that are mediated *via* the biotic trajectories T1 and T2 may also increase the proportion of^{13}C accumulated through the food chain. (b) Phylogenetically closely (left) and distantly related (right) neighbors may affect decomposition of litter of a focal tree *via* (i) above-ground processes affecting litter quality *via* abiotic and biotic mechanisms (microclimate, shared enemies, competition); and (ii) below-ground processes affecting the decomposition of a given litter quality *via* biotic and abiotic mechanisms (microclimate, decomposer community). (c) Experimental design: Transplantation litter between phylogenetically isolated and non-isolated trees permits to disentangle above-ground from below-ground effects.

Fig. 2. Effects of phylogenetic isolation aboveground and belowground (PIA and PIB) in multiple regression analysis on the mass loss, the C loss and the N loss of the oak litter (left panel); (b) the microbial biomass, the abundances of Acari and Collembola in the oak litter (right panel). Empty circle and dashed line stand for the effects of PIA, solid line and solid point stand for the effects of PIB. * indicates partial residuals. Significant effects ($P < 0.05$) are shown in regression line. r^2 represents the explanatory power of each model.

Fig. 3. Effects of phylogenetic isolation aboveground (PIA) and belowground (PIB) on carbon isotopes (^{13}C/^{12}C) of oak litter after 8 and 14 months according to multiple regression analysis. Empty circle and dashed line stand for the effects of PIA, while solid line and solid point stand for the effects of PIB. * indicates the partial residuals. Significant effects ($P < 0.05$) was shown in regression line. r^2 represents the explanatory power of each model.