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Positive feedback loop between earthworms, humus form and soil pH reinforces earthworm abundance in European forests

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Author contributions

HDW, KVM and BM designed the study; HDW, TD, FXJ and LV collected the data; ED, KVM, HDW analyzed the data; ED and KVM compiled the manuscript; All authors contributed critically to the drafts and gave final approval for publication.

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Data archive

Acknowledgements

Abstract

1. In many terrestrial ecosystems earthworms operate at the interface between plants and soil. As ecosystem engineers, they affect key ecosystem functions such as decomposition, nutrient cycling and bioturbation. Their incidence and abundance depends on several soil properties, yet simultaneously they also impact soil properties themselves. The existence of a positive feedback loop in which earthworm activity maintains their own niche - by promoting turnover rate in the forest floor, thereby increasing topsoil pH and creating suitable living conditions for themselves - has been suggested before, yet lacks supporting evidence.

2. Here we assessed how tree species litter traits relate to such belowground interactions in forests across Europe. Specifically, we hypothesized a belowground feedback loop between burrowing earthworm biomass, humus form and pH, affected by litter quality. We tested this hypothesis by means of structural equation modelling.

3. Our results demonstrate that litter nutrient concentrations affect both burrowing and litter dwelling earthworm biomass, which in turn directly impact humus form and indirectly soil pH. At a continental scale, i.e. including all edaphic conditions, soil pH did not feed into earthworm biomass nor could we link leaf structural recalcitrance (e.g. lignin) or functional diversity to belowground interactions.

4. However, in forests where moisture is not limiting, soil acidity proved an important factor determining the context of belowground interactions. Therefore, we were able to confirm the hypothesized feedback loop for forest ecosystems with soil pH ≤ 5. In calcareous and/or periodically dry forests, other factors than soil chemistry and litter quality became determinant for earthworm biomass.

5. The activity of burrowing earthworms is pivotal in belowground ecosystem functioning of mesic forest soils, impacting litter accumulation and forest floor conditions aboveground, the pH and nutrient status belowground and ultimately their own living conditions. This highlights earthworm bioturbation as a key mechanism for understanding plant soil-interactions in forests.

1. Introduction

Plant-soil interactions are increasingly examined to better understand the functioning of terrestrial ecosystems (van der Putten et al. 2013). Earthworms can play a crucial role in the mechanisms underpinning such plant-soil interactions as they are key agents active at the interface between plants and soil (Jones et al. 1994; Lavelle et al. 2006). They are affected by both aboveground vegetation characteristics and belowground conditions (Ponge et al. 1999; Schelfhout et al. 2017). Vice versa, these allogenic ecosystem engineers can also modify the environment in which they live and affect key soil processes including decomposition and nutrient cycling (Blouin et al. 2013), thereby contributing considerably to ecosystem functioning. Limited research has explicitly targeted if - and how - such engineering activity can impact earthworm abundance itself.

Since Darwin's pioneering work (Darwin 1881), a large number of studies have contributed to the understanding of the role of earthworms in their environment and the factors that affect them (Lavelle et al. 1997; Schelfhout et al. 2017). Previous work by De Wandeler et al. (2016) showed the impact of litter quality, understory vegetation and forest floor variables as drivers of earthworm incidence on a European scale. Soil chemistry has also been shown to influence earthworm occurrence and abundance: increasing soil nutrient availability and soil pH and decreasing exchangeable Al^{3+} concentrations, positively influence earthworm biomass (Bouché 1977; Cesarz et al. 2007; Mueller et al. 2016). The engineering effect by earthworms on their environment can be described by several cause-effect relationships (Jones et al. 2010). Earthworms induce structural changes in the soil: forest floor mass decreases by earthworms feeding on fresh litter or partly decomposed organic material (Satchell 1983; Haimi & Huhta 1990). In addition, soil structure is altered by their burrowing activities, fragmentation of organic material and their contribution to soil aggregation (Satchell 1983; Jones et al. 2010). Such structural changes may in turn cause other abiotic changes. Reduced forest floor accumulation and incorporation of organic matter in the soil leads to reduced production of strong organic acids (Haimi & Huhta 1990), and corresponds with a decrease in exchangeable soil aluminum (Schelfhout et al. 2017; Verstraeten et al. 2018) and associated increased

macro-nutrient availability (Reich et al. 2005). Given the ample evidence for these earthworm-induced changes in soil physicochemical properties, it has been suggested that they can have a positive feedback effect on the earthworm communities themselves (Ponge 2013). Specifically, earthworm-induced changes on soil pH may impact their own living conditions to such an extent that it subsequently impacts earthworm occurrence and abundance. However, so far this feedback has never been empirically demonstrated.

Individual cause-effect relationships between earthworms and soil physical or chemical properties have been observed in controlled experiments (Bohlen & Edwards 1995; Cesarz et al. 2016) as well as in the field (De Wandeler et al. 2016; Schelfhout et al. 2017). To fully understand the complex aboveground-belowground interactions and their impact on belowground functioning, we would ideally manipulate the different components of the feedback loop independently in controlled field experiments. However, this is not within reach as the processes involved might take decades to result in the conditions observed in real-world ecosystems. As an alternative approach, causal understanding can be derived from carefully designed observational studies by modelling the relationships between multivariate data using structural equation modelling (SEM) (Shipley 2000; Grace 2006). This tool, still underused in soil ecology, can help us to shed light on belowground interactions and understand the relationships at the aboveground-belowground interface (Eisenhauer et al. 2015).

We aimed to investigate the interactions between litter properties, humus form, soil geochemistry and earthworm abundance in the sites of the exploratory platform of the FunDivEUROPE project (Baeten et al. 2013). This platform consists of mature forest plots located in six geographical regions across Europe and was established to evaluate both tree species identity as tree species diversity effects on forest ecosystem functioning. Using SEM, we tested the hypothesized positive feedback loop between burrowing earthworms, humus form and soil pH on a continental scale: burrowing earthworms are a key driver of humus forms and quality (decreasing forest floor mass and formation of macro-aggregates in the topsoil), promoting nutrient turnover rate and increasing topsoil pH, which

in turn allows for suitable living conditions for most earthworm species and hence a higher earthworm biomass. In addition, we incorporated the aboveground ecosystem compartment in the SEM analysis by studying how tree litter traits and tree functional diversity influence the hypothesized positive feedback loop.

2. Material and methods

2.1 Study area

The study was performed in the exploratory platform of the FunDivEUROPE project (Baeten et al. 2013), which was specifically designed to assess biodiversity-ecosystem function relationships along a tree species richness gradient in mature forests. The six studied regions in this platform span most of the European bioclimatic gradient and represent major European forest types including boreal forest (North Karelia, Finland), hemiboreal forest (Białowieża, Poland), temperate beech forest (Hainich, Germany), mountain beech forest (Râșca, Romania), thermophilous deciduous forest (Colline Metallifere, Italy) and Mediterranean-mixed forest (Alto Tajo, Spain). Each region included between 28 and 43 plots (30 m x 30 m) with different combinations of a fixed set of locally dominant tree species. The established plots ranged in tree species richness from one to three, one to four or one to five species per plot (depending on the region). In total, the platform consisted of 209 plots with 16 target tree species, some of them occurring in multiple regions. The species pool comprised evergreen conifers, deciduous broadleaves and evergreen broadleaves. For more details consult Baeten et al. (2013).

2.2 Data collection

Tree leaf litter traits

In all 209 plots, tree leaf litter was collected with 5 litter traps per plot, oven dried and used to determine tree leaf litter biomass per species per plot. Tree species-specific leaf C and N concentrations, C:N ratio and calcium (Ca) concentration in addition to fourteen leaf litter traits (Table S1) were determined from freshly fallen leaf litter of each species, collected at several locations per region at peak leaf litter fall between October 2011 and November 2012 (for detailed methods see

Joly et al. (2017)). Tree basal area-based community weighted means (CWM) for all 18 leaf litter traits were calculated per plot based on the species-specific litter traits and plot-specific annual litterfall. Functional dispersion (FDis) was calculated as a proxy of tree functional diversity and measures the weighted mean distance in a multivariate functional trait space to the weighted centroid of all tree species (Laliberté & Legendre 2010). High values of FDis indicate that the tree species are more functionally distant from one another.

Forest floor

The forest floor in this manuscript refers to the ectorganic layer and comprises three organic horizons: the OL, OF and OH horizon. In the center of each plot, humus form was assessed based on the European Reference Base for Humus Forms (Zanella et al. 2011). The type of A horizon (biomacro-, biomeso-, and biomicro-structured, single grain, massive) and the incidence of a humified forest floor layer, i.e. OH horizon, were visually assessed in the field (presence vs. absence). The forest floor thickness (cm) was measured and additional samples were taken to determine the dry mass (kg/m²) and C:N ratio of the forest floor. For more details on the assessment of the forest floor thickness, mass and C:N ratio, see De Wandeler et al. (2016).

Earthworm biomass

Earthworm sampling was carried out in Spring 2012 for the regions in Italy, Germany and Finland, and in Autumn 2012 for the regions in Poland, Romania and Spain, as humid soil conditions and positive temperatures are recommended during sampling (Eggleton et al. 2009). Earthworms were sampled in one point in the center of each plot by means of a combined method. First, litter was removed and hand sorted over an area of 25 x 25 cm. Then, litter was removed in the same way, but over a larger area of 1 x 0.5 m surrounding the hand sorted area. On the denuded soil of that larger area, an ethological extraction of earthworms from the soil using a mustard suspension was performed (according to Valckx et al. 2011). Finally, digging out and hand sorting of a soil sample of 25 × 25 cm and 20 cm depth was performed in the middle of the 1 x 0.5 m area. Collected earthworms were preserved and all earthworms were individually weighed, including gut content, upon identification.

Results per unit area of the three sampling techniques were summed to determine the earthworm biomass per m². Earthworms were subsequently divided into two functional groups based on their bioturbation capacity: litter dwellers (epigeics) and burrowers (comprising both endogeics and anecics) (Sims & Gerard 1999; Ponge et al. 1999) (Table S10). We acknowledge that endogeic and anecic species portray different feeding behavior however in terms of bioturbation their role is comparable in comparison to the smaller epigeic species that miss the muscular power and force to mix organic matter and mineral soil and that live on top of the soil. For more details consult De Wandeler et al. (2016).

Soil properties

In each plot, a composite sample of 9 subsamples of the mineral soil (0-10 cm) was collected (Dawud et al. (2016; 2017). Soil pH (CaCl$_2$) was determined with 0.01 M CaCl$_2$ solution at a ratio of 1:2.5, using 827 pH lab (Metrohm AG, Herisau, Switzerland) after the soil was dried to constant weight (55°C) and sieved through a 2 mm diameter mesh. Texture (sand, silt and clay percentage) was determined using a laser diffraction particle size analyzer - LS 13 320 after pre-treatment (ISRIC). The volumetric stone content of the soil was estimated with the metal rod method by Viro (1952) and the empirical equation presented by Tamminen & Starr (1994).

2.3 Statistical analysis

All statistical analyses were done using the R statistical software (R Core Team 2019). From the 209 plots, there were four plots with partially missing earthworm biomass data (i.e. no distribution of total biomass to functional groups) and one plot with missing humus data. To be able to use all observations in our statistical analyses, we used predictive mean matching, implemented in the mice R package (Buuren & Groothuis-Oudshoorn 2011) to impute these missing values based on the variables included in the SEM model.

In order to reduce the complexity of the final structural equation models, we first extracted the most important gradients in litter trait and forest floor properties using a principal components (PCA) and principal coordinates analysis (PCoA), respectively. The PCA was performed on the correlation matrix

of the litter traits using the vegan package (Oksanen et al. 2019). The scores of the first axis were used as a single indicator of leaf litter nutrient content (Figure 1, Table S1) and the scores of the second PCA axis were used as a proxy for structural recalcitrance (Table S1). The dimension of forest floor properties (quantitative, ordinal and nominal) was reduced through a Gower dissimilarity-based PCoA, performed with the ape package (Paradis & Schliep 2018), with the Cailliez procedure to correct for negative eigenvalues (Calliez 1983). The first PCoA axis was used as an indicator for humus form (Figure 2, Table S2).

In a next step, the separate links between earthworm biomass (transformed to log(biomass+0.1)+1 according to McCune and Grace, 2002), litter, humus form and soil properties were explored by linear mixed models (LMM) including site as a random factor (Table S4). Because of the many zero values present in the earthworm biomass data, all relations with biomass as a response variable were modelled with zero-inflated-mixed models (ZIMM) with Gaussian error distribution using the NBZIMM package. The estimates and P-values of the ZIM models did not differ considerably from the estimates and P-values of linear mixed models (Table S5). Earthworms are known to show non-linear behavior over an acidity gradient (Muys 1995), therefore we used generalized additive mixed modelling (GAMM) to examine this relationship more closely (Figure 4, Table S4). The GAMM was performed with the mgcv package using penalized regression splines taking litter and soil variables into account as covariates and region as random factor. These bivariate relationships were then used to build the structural equation model with an additional separation of earthworm biomass per functional group.

Finally, we used structural equation modelling (SEM, Eisenhauer et al. 2015; Grace 2006) to quantify the relationships between tree leaf litter, earthworm biomass, humus form and mineral soil pH. These relationships are investigated on a continental scale across the six sampled regions and their tree diversity gradients. To start the SEM analysis, we created a conceptual meta-model where we connected all separate compartments and hypothesised that there exists a positive feedback loop between earthworms, humus form and soil pH.Burrowing earthworms contribute to change the

humus form (decreasing forest floor mass, well-structured A-horizon as a consequence of incorporation of organic matter into the mineral soil through bioturbation) that in turn increases pH of the topsoil, thereby allowing a more diverse earthworm community in the soil and a higher burrowing earthworm biomass. The rationale behind every hypothesized path is described in Table 1. Based on the *a priori* specified causal relationships, we built a SEM by populating each component of the meta-model with our measured variables (Figure 6a). The linearity of the proposed relationships was first investigated. As the GAMM analysis of the pH-earthworm relationship showed a clear threshold around pH = 4.5 (Figure 4), two versions of the SEM were fit: one for all plots and one for the observations with a soil pH ≤ 5. The statistical decision was made to take pH of 5 as a cut-off as to have a linear significant relation and to retain enough data points for the SEM analysis.

The number of clusters (6 regions) was too low in order to be able to perform a multilevel SEM (McNeish & Stapleton 2016) and the number of parameters was too high in relation to the number of observations to allow for an accurate multigroup SEM model fit or to fit 6 separate SEMs. Therefore, we accounted for the hierarchical structure in the data (plots within 6 regions) by first regressing all model variables on region identity (y ~ region) to remove the region effect and execute all subsequent analyses on the residuals. This method removed all between-site variation (e.g. macroclimate) from the data.

The SEMs were fit using the 'lavaan' package (Rosseel 2012). Significance and goodness-of fit of the final model were assessed using a combination of model fit indices: (i) the model chi-square with a non-significant P-value (> 0.05) indicating an overall good model fit (H_0: the model-implied covariance matrix equals the observed covariance matrix), (ii) the Comparative Fit Index (CFI), which is little affected by sample size, with a value of >0.95 indicating a good model fit, (iii) and the Root Mean Square Error of Approximation (RMSEA) where a value ≤ 0.06 can be considered as a good fit (Hooper et al. 2008). Finally, each link in the final model was evaluated for significant contribution to the model and standardized coefficients and the explained variance per response variable were calculated.

Because zero-inflated models with Gaussian error distribution cannot yet be incorporated in the mgcv and lavaan packages, the excess zeros in the dataset could not be taken into account in the GAMM and SEM analyses. Still, we consider our approaches appropriate for several reasons. First, the GAMM is used as an exploratory analysis to reveal non-linear relationships in the data and to guide the SEM models. Second, we compared the univariate ZIMMs with the regular LMM (without zero-inflation parameters) and their results (estimates and P-values) did not differ considerably (Table S5). And third, we checked the residuals of the SEM (fitted – observed responses) and they were normally distributed (Figure S4).

Table 1: The rationales behind every hypothesized path included in the SEM. The meta-model (Figure 6a) was built based on the described causal relationships. The relation between two variables can only be included in one direction (e.g. soil pH ~ humus form).

		Responses			
		Burrowers	Litter dwellers	Humus form	Soil pH
Predictors	Litter nutrients	Higher? Tree leaf litter nutrient concentration positively stimulates earthworm biomass (Reich et al., 2005).		Independent from earthworm activity, high leaf litter nutrient concentration increase litter decomposition (Hättenschwiler, 2005), which results in a decreasing mass of the ectorganic layer and a mull-type humus form.	
	Litter recalcitrance	Leaf litter recalcitrance has a negative influence on earthworm biomass, since recalcitrant litter is disliked, hard to digest by earthworms and proven to have a negative impact on their growth (Ponge, 2013; Kasurinen et al., 2007).		Recalcitrant litter is hard to digest by most soil organisms (Ponge 2013) and will slow decomposition leading to forest floor build-up and a mor humus form.	
	Litter functional dispersal	Tree species functional dispersion positively influences earthworm biomass by increasing the diversity of litter types and habitat availability. The magnitude of the effect is very small in comparison to tree species identity effects (De Wandeler et al. 2018).		Independent from earthworms, litter functional dispersion positively affects humus form by increasing the diversity in food types and habitat availability of other litter dwellers and decomposers (Hättenschwiler et al., 2005).	
	Litter dwellers and Burrowers			Earthworms determine to a large extent the humus form present by feeding on fresh litter or partly decomposed organic material and thereby decreasing the forest floor mass. HIgher earthworm biomass will change the humus form (from mor to mull).	We hypothesize that soil pH is **indirectly** affected by earthworm biomass via the impact of earthworms on humus form.
	Burrowers	We hypothesize ... **indirectly** reinforce earthworms via their impact on humus form and again on soil pH.		Burrowers mix the topsoil layer and consequently contribute to a well-structured A-horizon. A decrease in biomass leads to forest floor build-up, acidifies the forest floor which results in the presence of an OH horizon and a change in humus form (Zanella et al. 2011).	
	Humus form				Build-up of the forest floor and changes towards a mor-like humus form will lead to the production of more acidic intermediate decomposition products and thereby lower the pH of the mineral soil below (Zanella et al. 2011).
	Soil pH	Soil pH influences burrowing earthworm incidence and biomass directly since high exchangeable Al^{3+} concentrations and low pH values inhibit earthworm growth and cocoon production (van Gestel and Hoogerwerf 2001).	Epigeic earthworms are not affected by soil pH because: (1) they do not inhabit the soil and (2) they are more tolerant to acidic conditions.	Low soil pH prevents microbial decomposition and decreases nutrient availability in the soil (Ott et al. 2014) negatively affecting all soil fauna (including earthworm biomass) leading to a mor-type humus form. Because the impact of humus form on soil pH > soil pH on humus form (independent from earthworms) we only included the first in the SEM.	
	Clay				Clay is a good proxy for acid buffering capacity and thereby positively related to soil pH.

3. Results

3.1. Litter properties

The principal components analysis (PCA) on all litter properties over all regions resulted in a litter nutrient (PC1) and a litter structural recalcitrance axis (PC2) explaining respectively 39% and 25% of the variation in litter trait space (Figure S1). PC1 was strongly correlated with litter macronutrient concentrations and litter C/N ratio. The second axis was positively correlated with lignin concentration of the leaf litter (Figure 1, Table S1). The litter nutrient concentration PC showed a positive relation with earthworm biomass of both functional groups whereas no significant relations between earthworm biomass and litter structural recalcitrance or functional dispersion were found (Figure 1, Table S4).
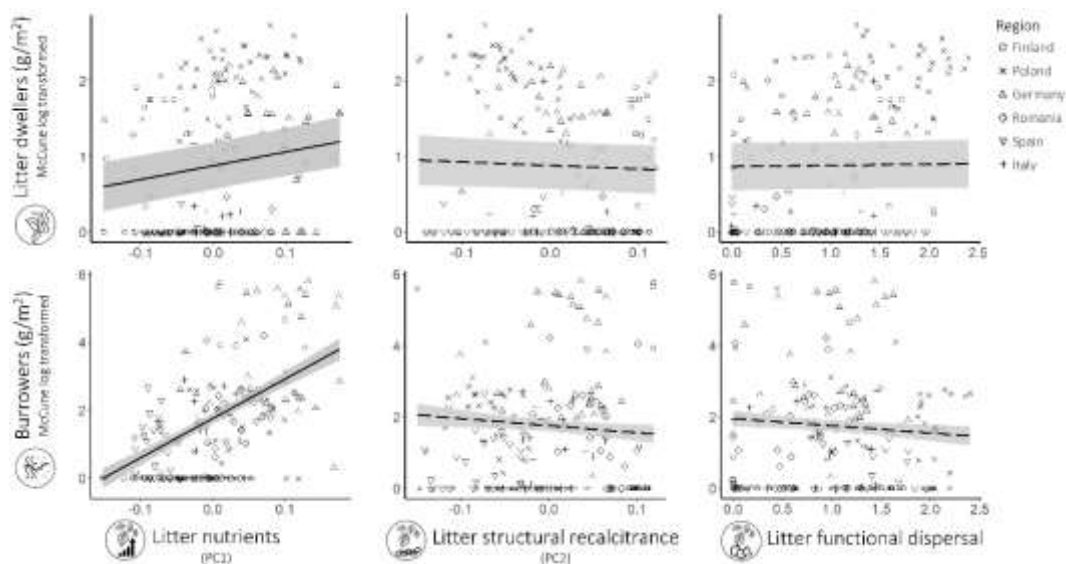

Figure 1. Earthworm biomass (transformed to log(biomass+0.1)+1 according to McCune and Grace, 2002) per functional group (litter dwellers and burrowers) as a function of litter nutrient content (axis 1 of PCA in figure S1), litter structural recalcitrance (axis 2 of PCA in figure S1) and litter functional dispersion. Relations are tested using zero-inflated mixed models and significant relations are indicated by a full line whereas non-significant relations are indicated by a dotted line (table S4). The shaded parts indicate the standard error interval (± one SE). Observations are indicated per region; Finland (circles), Poland (x's), Germany (triangles), Romania (diamonds), Spain (triangles) and Italy (+'s).

3.2. Humus form

All four basic terrestrial humus forms, i.e. mull, moder, mor and amphi, were observed across the investigated 208 forest plots. A PCoA analysis based on humus characteristics showed two gradients explaining respectively 60% and 13% of the variation in humus forms on a European scale (Figure 2).
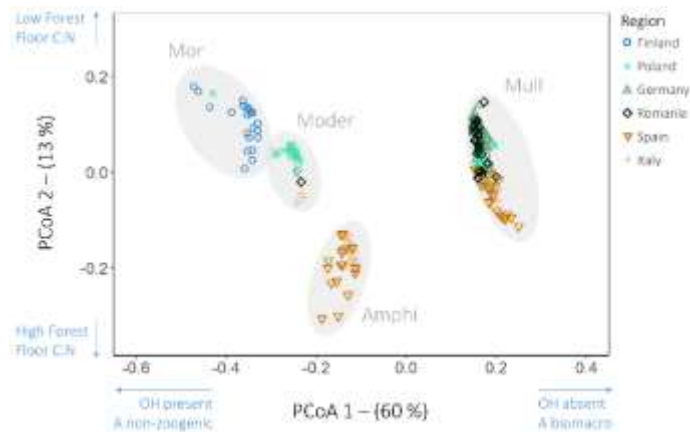

Figure 2. Principal Coordinate Analysis (PCoA) biplot of the 208 sampled forest plots based on humus form characteristics. The grouping of the plots according to the four basic humus forms is indicated in green. Information of the variables that showed the highest correlation with the first two PCoA axes (OH horizon presence/absence, A horizon structure and Forest Floor C:N) are shown in blue along the axes. More details on the humus forms and the correlation coefficients can be found in Table S2. Observations are indicated per region; Finland (blue circles), Poland (green x's), Germany (green triangles), Romania (dark diamonds), Spain (orange triangles) and Italy (yellow +'s).

The first axis (PCoA1) illustrates that humus form ranged from mor, with a clear OH horizon and a poorly aggregated A horizon with no or hardly any zoogenic bioturbation activity, to mull, without OH horizon and a well-structured A horizon developed from soil fauna activity. On the second axis (PCoA2) the difference between the humus forms from the Eurosiberian ecoregion with cool humid climates (mull, moder and mor) and amphi humus forms from the Mediterranean ecoregion with summer drought conditions is visible. Both PCoA axes had a significant positive relationship with total earthworm biomass. Within the functional groups of earthworms PCoA1 was correlated with both biomass of burrowers and litter dwellers whereas PCoA2 was only correlated with burrowing earthworms biomass (Figure 3, Table S4).
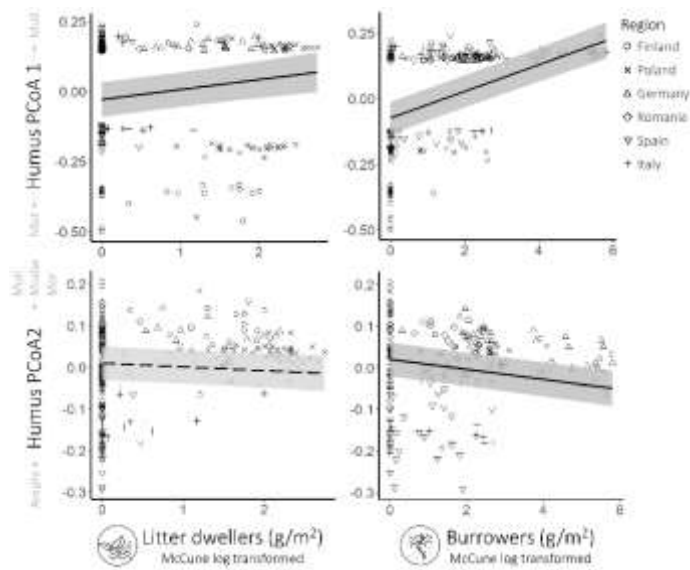
Figure 3. Humus form characteristics (PCoA1 and PCoA2) as a function of earthworm biomass (transformed to log(biomass+0.1)+1 according to McCune and Grace, 2002) per functional group (litter dwellers and burrowers). Full lines indicate significant relations whereas dotted lines indicate relations that are not significant. The shaded parts indicate the standard error interval. Observations are indicated per region; Finland (circles), Poland (x's), Germany (triangles), Romania (diamonds), Spain (triangles) and Italy (+'s).

3.3. Soil properties

For both clay content and topsoil pH, no significant linear relation was found with total earthworm biomass or per functional group (Figure S2, Table S4). However, when using generalized additive mixed modelling, we found a non-linear relation between burrowing earthworm biomass and soil pH: biomass increased with increasing soil pH (Figure 4, Table S4). The relationship between soil pH and burrowing earthworm biomass was positive only up to a threshold at pH 4.5 – 5 , thereafter the relationship became slightly negative (Figure 4). Texture (clay content) could not be significantly related to earthworm biomass using linear or non-linear methods (Figure 4, Figure S2, Table S4).
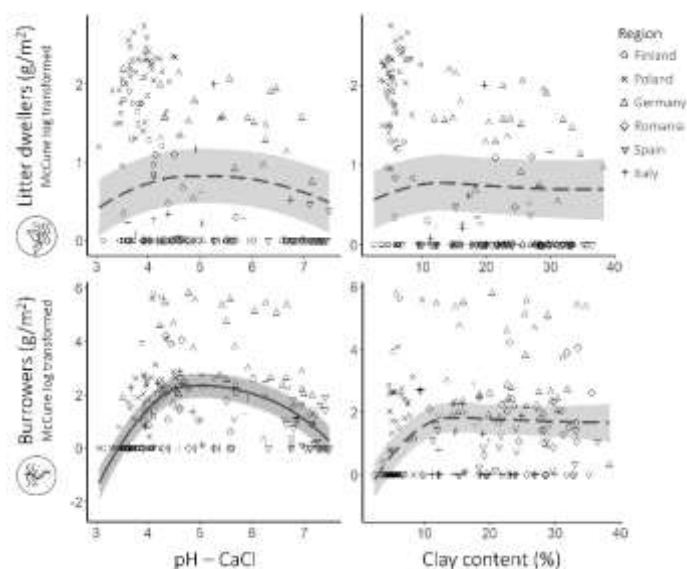
Figure 4. The GAMM model explaining earthworm biomass per functional group (litter dwellers and burrowers) based on topsoil pH (left) and clay content (right). The model shows the relationship between biomass (transformed to log(biomass+0.01)+1 according to McCune and Grace, 2002) and soil pH or clay content taking into account the influence of litter nutrients, litter structural recalcitrance, stoniness and clay or pH respectively. The shaded parts indicate the standard error interval (± one SE). Full lines indicate significant relations whereas dotted lines indicate relations that are not significant. Observations are indicated per region; Finland (circles), Poland (x's), Germany (triangles), Romania (diamonds), Spain (triangles) and Italy (+'s).

3.4. Structural equation models

The structural equation model including data points spanning the whole pH range, fitted the data well (Chi-square test statistic = 11.51, df = 8, p = 0.17, CFI = 0.97 and RMSEA = 0.05, Figure 5, Table S6). Leaf structural recalcitrance and leaf litter functional dispersion did not significantly affect the characteristics of the soil compartment. Soil pH is positively affected by clay content of the soil and by humus form. The *a priori* hypothesized link from burrowing earthworms to humus form to soil pH and back to the biomass of burrowers was not confirmed (p = 0.73). However, these same links were found significant in a second SEM that only included data points with a pH ≤ 5 (Chi-square test statistic = 13.40, df = 8, p = 0.09, CFI = 0.96 and RMSEA = 0.07). In these acidic soils (from oligotrophic to mesotrophic), the path from earthworm biomass to humus form and further to soil pH, which is then linked back to the biomass of burrowing earthworms, was confirmed (p = 0.002). This supports the hypothesized positive feedback loop between burrowers, humus form and soil pH for acidic soils

(Figure 5, Table S7). In the SEM for pH≤5 the direct relation from litter nutrients to litter dwellers was no longer significant (P=0.11). However when we accounted for the zeros using zero-inflated models (Table S5) it remained significant (P=0.004). The fit of alternative models, e.g. with a reversed feedback loop (Table S8) or no feedback loop (Table S9), was considerably lower compared to the illustrated model in Figure 5.

Table 2. The direct, indirect and total standardized effects of litter nutrients, burrowers, litter dwellers, humus form, pH and clay on respectively burrowers, humus form, litter dwellers and soil pH for SEM pH ≤ 5 (Chi-square test statistic = 14.06, df = 8, P = 0.08, indicating a good fit, see materials and methods).

Predictor	Pathway to response:	Burrowers	Humus form	Litter	Soil pH
Litter nutrients	*Direct effect*	*0.273*	*0.155*	*-*	*-*
	Indirect via burrowers	*0.006*	*0.132*	*-*	*0.024*
	Indirect via humus form	*0.007*	*-*	*-*	*0.028*
	TOTAL	0.286	0.287	-	0.052
Burrowers	*Direct effect*	*-*	*0.483*	*-*	*-*
	Indirect effect	*0.023*	*-*	*-*	*0.088*
	TOTAL	0.023	0.483	-	0.088
Litter dwellers	*Direct effect*	*-*	*0.188*	*-*	*-*
	Indirect effect	*0.009*	*0.004*	*-*	*0.034*
	TOTAL	0.009	0.192	-	0.034
Humus form	*Direct effect*	*-*	*-*	*-*	*0.182*
	Indirect effect	*0.048*	*0.023*	*-*	*0.004*
	TOTAL	0.048	0.023	-	0.186
pH	*Direct effect*	*0.266*	*-*	*-*	*-*
	Indirect effect	*0.006*	*0.128*	*-*	*0.023*
	TOTAL	0.272	0.128	-	0.023
Clay	*Direct effect*	*-*	*-*	*-*	*0.363*
	Indirect effect	*0.096*	*0.047*	*-*	*0.008*
	TOTAL	0.096	0.047	-	0.371

The direct, indirect and total standardized effects were indicated by the coefficients (reported for each predictor in Table 2). For example, the indirect effect of clay on burrowing earthworm biomass was 0.096 (i.e. the product of the separate pathways). This effect was larger than the indirect effect of humus form on burrowing earthworm biomass (0.048) or the effect that burrowing earthworms had on themselves (0.023).
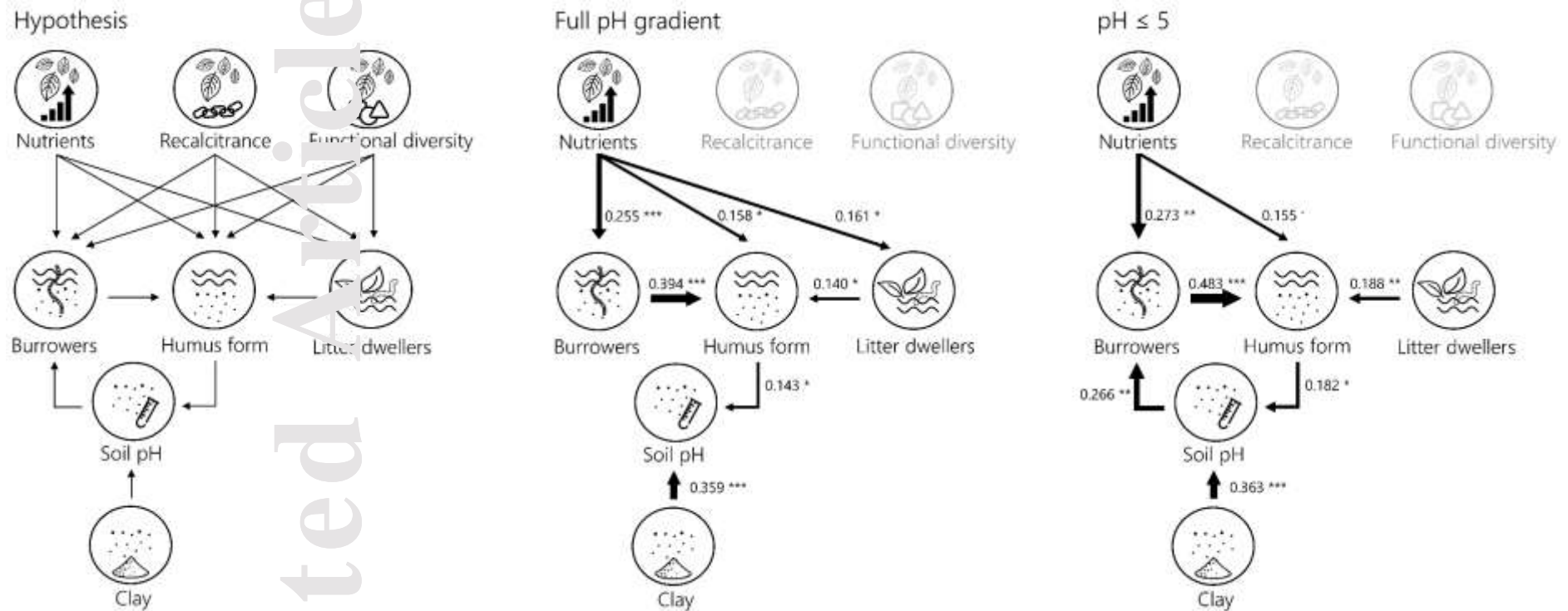
Figure 5: Left: The a priori causal ...... model showing all the relationships tested. Leaf litter traits (nutrient concentration, structural recalcitrance and functional dispersion) are linked to the forest floor (hurus form) and earthworm functional groups (burrowers and litter dwellers). We hypothesize a feedback look from burrowing earthworms to humus form to soil pH and back to burrowing earthworm biomass (Table 1). Middle: model across all regions based on the entire pH gradient (Chi-square test statistic = 11.52, df = 8, P = 0.17). Right the model on observations with pH values ≤ 5 (Chi-square test statistic = 13.40, df = 8, P = 0.09). Numbers next to arrows give standardized path coefficients with their significance indicated as ***P < 0.001, **P < 0.01, *P < 0.05, · P = 0.05. Solid arrows represent positive effects, dashed arrows represent negative effects (which are not present), non-significant relations are not displayed. Arrow width is proportional to the size of the standardized model coefficients.

4. Discussion

Earthworms reinforce earthworms

Our study provides evidence that earthworm activity modifies soil properties in a way that reinforces earthworm biomass. In oligotrophic to mesotrophic soils (pH ≤ 5) we found a strong connection between burrowers, humus form and soil pH that can be interpreted as a positive feedback loop with burrowing earthworms affecting humus form which in turn impacts earthworm biomass via higher mineral soil pH. In this feedback loop, bioturbation by burrowing earthworms is an essential ecosystem function counteracting organic matter accumulation and related organic acid production at the soil surface, and can be considered a widespread example of allogenic ecosystem engineering, as defined by Jones (1994). In ecosystems where burrowing earthworms are absent, fresh organic matter is not incorporated in the soil through bioturbation, leading to the accumulation of organic matter in the forest floor and the corresponding production of strong organic acids as intermediate decomposition products, ultimately lowering the soil pH (Ulrich & Sumner 1991). This highlights the importance of soil fauna-mediated organic matter decomposition on soil pH (Haimi & Huhta 1990; Schelfhout et al. 2017). The magnitude of the indirect effect of burrowing earthworms on themselves is, however, modest in comparison to the direct effect of litter nutrient concentrations, soil pH or even the indirect effect of texture and humus form.

The earthworm-pH relationship has been suggested manifold (Bouché 1977; Augusto et al. 2015; Reich et al. 2005) and some studies have even suggested a reinforcing feedback loop (Ponge 2013; Desie et al. 2019). However, when these studies in the past failed to document such relationships, it was probably because of the assumed linearity. A typical threshold relationship between soil pH and burrowing earthworm biomass was found with a positive linear trend until approximately a pH of 4.5 (Figure 4). Above this threshold, larger-sized burrowing species (endogeic and anecic) dominate, whereas below this threshold more acid tolerant species survive (De Wandeler et al. 2016). In terms of burrowing activity below the threshold: acid sensitive anecic species like Aporrectodea longa or Lumbricus terrestris are no longer present and more acid tolerant epi-anecic species, such as

Lumbricus rubellus, in combination with slightly acid tolerant humus-feeding endogeic species, such as Octolasion spp., rework the soil (see Muys & Granval 1997 for pH ranges of earthworm species). This is also illustrated in our study: significant relations between burrowing earthworm biomass and topsoil pH and C:N ratio suggest that it is mostly burrowers that are susceptible to the chemical conditions of their environment. In forest ecosystems with well-buffered soils (pH > 5), soil pH had no effect on earthworm biomass, which suggests that other factors such as moisture and litter availability become the main drivers of earthworm biomass (Eggleton et al. 2009). The slightly decreasing earthworm biomass for higher soil pH values is probably no soil pH effect, but an indication of aridity in the calcareous soils of the Mediterranean study region in Spain, which causes the formation of amphi humus forms, that are less favorable for earthworms than mull humus. Sites with high soil pH are very often confounded with semi-arid sites (Slessarev et al. 2016) and, consequently, this study only included few soils with high pH values and no water limitation. For soils located in arid regions (e.g. Spain and Italy) local climate and water availability become driving factors of belowground communities (De Wandeler et al. 2016). This could however not be formally tested here, because of the lack of within-regional variation in local climate and because plot-level data on water availability were not available.

The division at pH 4.5 corresponds with a pedogenic threshold in acid buffering, where soil processes are either dominated by iron and aluminum or by base cations (Vitousek & Chadwick 2013). This pedogenic threshold goes beyond soil chemistry and was previously associated with two different ecosystem states where not only earthworm activity changes considerably, but also soil nutrient cycling, microbial diversity and carbon sequestration (Ponge 2013; Desie et al. 2019). If mechanisms differ on either side of the pH threshold of 4.5, it is nearly impossible to capture belowground feedbacks when modelling the full pH range. Interestingly, Slessarev et al. (2016) demonstrate the existence of a global threshold around a pH of 5 created by water balance; if the mean annual precipitation (MAP) is higher than the potential evotranspiration (PET) the excess of water leaches nutrients from the soil and over time leads to more acidic conditions. This means that globally there

are more acid soils compared to soils with a neutral pH (between 6 and 7) and it is in such acidic soils that the discussed feedback loop becomes active.

Evaluation of a feedback loop using structural equation modelling ideally involves a temporal component to evaluate the dynamics of change. In our study we evaluated relations in mature forest stands that can be assumed to be in steady state, based on single-point-in-time data, and could not evaluate the actual changes in belowground functioning over time. As mentioned by Grace (2006), estimation of such non-recursive models can lead to complications with model identification. However, the underlying causal structure of our SEM did not change and independence of the predictors allowed model identification, therefore we are confident about our findings. Moreover, the statistical interpretation, i.e. the three strong relations that are connected, should be seen separately from the ecological interpretation of the feedback loop, which implies a dynamic mechanism. We see it as an advantage that our study is based on mature forest stands in steady state since it allowed for evaluation of long-term effects of earthworms on belowground functioning that would be hard to evaluate in short-term manipulated ex-situ experiments (Barot et al. 2007).

Plant-soil interactions

Aboveground and belowground communities are intrinsically linked and interactions between both subsystems have major implications for ecosystem functioning (van der Putten et al. 2013). The humus layer is the place where aboveground and belowground communities interact and therefore it also forms the seat of feedbacks between these communities (Ponge 2013). The earlier discussed positive feedback loop in the belowground compartment of forest ecosystems is partly driven by aboveground factors such as tree species composition and by the impact of these factors on earthworm biomass (Wardle et al. 1997).

Although the food web theory predicts increased biomass of higher trophic levels with increased producer diversity (Cardinale et al. 2011), tree diversity effects on earthworm biomass seem negligible in comparison with tree species identity effects. As previously reported, tree identity is a

more important driver of earthworm biomass (Schwarz et al. 2015), litter decomposition (Joly et al. 2017), topsoil chemistry and soil carbon stock (Dawud et al. 2016, 2017) as compared to tree species diversity. Within the tree species identity effects, only leaf litter nutrient concentration was found to have an effect on earthworm biomass and humus form. Leaf litter nutrient concentrations, particularly Ca, promote higher earthworm biomass by supporting the Ca requirement in the calciferous glans of litter feeding earthworms (Piearce 1972; Reich et al. 2005). A recent study also suggested the greater importance of leaf litter nutrient contents, in comparison to soil C/N ratio, especially in forests where base cations have become limiting as a consequence of acidification and/or of high loads of N through atmospheric deposition (Desie et al. 2020).

The direct effect of leaf litter nutrients on humus form corresponds with the positive impact of litter quality on other soil fauna and decomposers. Moreover, leaf litter nutrient concentrations positively affect both litter dwelling and burrowing earthworm biomass which further impact the humus form and the soil chemistry. The humus form is the result of decomposition and incorporation of organic material into the soil in which several actors, like earthworms, arthropods, bacteria and fungi, play their part. In this European study, humus form can be explained by two gradients: a gradient of decomposition and bioturbation with humus forms ranging from mull to mor, and second, a gradient in forest floor C/N ratio that differentiates the temperate humus forms from the amphi humus forms in the drier southern-European regions. The humus form in temperate forests was significantly influenced by all functional groups of earthworms. This corresponds with Ponge (2003), who discussed how earthworm community (functional) composition is determinant for humus form: in order to find mulls it is essential to have both fast decomposition and subsequent rapid incorporation of fresh material into the mineral soil by burrowers (Zanella et al. 2011). If a burrowing community is absent, the result is a moder humus form, where bioturbation by epigeic earthworms and mesofauna like micro-arthropods is limited (Ponge 2003). If furthermore epigeic earthworms are near to absent, decomposition becomes dominated by microbiota instead of meso- and macrofauna leading to limited vertical distribution of organic matter, i.e. humus forms are classified as mor (Ponge 2003).

The gradient in forest floor C/N-ratio shows a distinction between amphi humus forms and humid temperate humus forms (Figure 3). This second gradient was significantly influenced by burrowing earthworm biomass whereas litter dwellers have no impact. In amphis, the superficial, periodically very dry organic horizon is generally not inhabited by earthworms, restricting their presence to the deeper soil horizons (De Nicola et al. 2014). Because mainly burrowers are active, an amphi humus form, although having a high forest floor C:N ratio, is most related to a mull humus type (Zanella et al. 2011). The high ambient temperatures lead to (1) higher metabolic activity and faster decomposition and also to (2) higher potential evapotranspiration and decreased organo-mineral migration into the mineral soil (resulting in less leaching of nutrients and less probability of acidified soils).

Finally, discussing two opposing scenarios (as examples) allows to illustrate the pervasive effect that litter quality can have on belowground functioning. Example 1: A change in overstory tree composition (e.g. broadleaved to coniferous tree species) leads to the dominance of litter with low nutrient concentrations in a forest with a mull humus form, all functional groups of earthworms present and soil pH around 5 (soil processes are buffered by base cations), yet with low acid buffer capacity due to the low clay content. In the short-term earthworms are affected by the change in litter quality. The humus form will change (less incorporation in the soil, more build-up of OH layer, less aggregation of topsoil) and more acidity will be produced in the accumulating forest floor (van Breemen et al. 1983). When the acid buffering range is exhausted, soil pH will decrease, leading to less favorable conditions for earthworms, reinforcing the negative trajectory. In the long-term a new steady state will develop: a mor humus type, soil pH approaching 3 and no earthworms. Example 2: A change in litter quality to higher nutrient concentrations in a forest with a mor/moder humus type, only epigeic earthworm species present and soil pH below 4. If there is no dispersal limitation, endogeic species are promoted by the nutrient rich litter. The humus form will improve due to the burrowing activity. Less acidity will be produced in the forest floor and nutrients are well distributed vertically leading to an increase in pH, favoring conditions for burrowing earthworm species. Once that pH is 5 or higher, soil pH does no longer significantly, linearly, affect the biomass of burrowers

and the feedback is no longer active. The effect of earthworms on soil pH remains present but it no longer reinforces their abundance. Thus, overstory composition and its associated litter quality can have a profound impact on belowground communities directly and indirectly by strengthening a feedback loop in the belowground ecosystem. Tree species functional identity is thus not only a strong driver of aboveground functioning (De Groote et al. 2017), but also strongly regulates the functioning within the belowground compartment.

Conclusion

The positive feedback loop between earthworms, humus form and soil pH, here illustrated for acidic soils (from oligotrophic to mesotrophic), improves our understanding of soil earthworm-mediated plant-soil interactions and belowground functioning in European forests. Our results highlight how earthworms act as ecosystem engineers and to what extent they can impact their environment. Different functional earthworm groups play either minor or major roles depending on local climate, soil type and the quality of input via aboveground community composition. In drier forest ecosystems, local climate properties and water availability drive decomposition and nutrient cycling; decomposition becomes more dominated by microbiota and earthworms play only a supporting role. However, on a continental scale many forests (humid temperate, boreal and tropical) are characterized by rather acidic soils, certainly in regions where there is an overall rainfall surplus. In such forest systems where soil pH is lower than 5, the activity (or inactivity) of burrowing earthworms drive belowground ecosystem functioning, impacting what happens on top of the soil, the chemical and nutrient status in the soil and ultimately their own living conditions. Moreover, our study illustrates how leaf litter nutrient concentrations impact earthworms and thereby also the feedback loop. Collectively, our study suggests a belowground feedback loop between earthworms, humus form and soil pH that reinforces earthworm abundance in acidic European forests and thereby also indicates the far-reaching impact that aboveground tree species composition can have on belowground functioning.

Previous research has evidenced the separate relations of the feedback loop in both short-term ex situ experiments and observational studies. Here, we evidence the strong connection between three relationships that could act reinforcing. Future research should combine such efforts in long-term in-situ experimental approaches to contribute to the growing body of evidence for the earthworm feedback loop. Adding a temporal component by manipulating the three variables in the feedback loop and monitoring the changes in belowground functioning would provide additional understanding of the causality, magnitude and time scale of the involved effects.

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