

Land use intensification results in abrupt transitions between contrasting grassland states

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Abstract

Understanding whether land use intensification causes regime shifts is of key importance for management, particularly if these shifts are associated with thresholds separating different ecosystem states and with hysteretic dynamics. Here we use a unique, long-term grassland database to identify thresholds in the response of 16 ecosystem functions and the diversities of 21 taxa to land use intensity. We show that aboveground diversity (5 of 10 taxa), shoot biomass and soil N retention showed threshold responses to land use intensity, i.e., abrupt changes between extensively and intensively managed grasslands. Time-series analysis revealed that ecosystem functions showed hysteresis around the threshold, while diversity did not. Shifting back to the functioning seen in extensively managed grasslands may therefore require larger reductions in land use intensity than shifting to the high intensity state. Identifying such thresholds along land use gradients is critical to prevent ecosystem degradation and conserve biodiversity and ecosystem functions.

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Statement of authorship

HS, LN, CP and EA designed the project. KB, NB, SB, MB, FB, AMFD, MF, KG, MG, UH, NH, KJ, EK, VHK, TK, SL, SM, YO, JO, DP, SR, SS, MS, IS, JS, SS, ES, ISD, BS, CW, WW, TW and SW collected the data. HS and CP assembled the dataset. HS analysed the data, HS, CP and EA interpreted the results and wrote the first draft of the manuscript. All authors discussed and commented on the final version of the manuscript.

Data accessibility statement

All data is currently available in <https://www.bexis.uni-jena.de/>, within the dataset with id 27087 and 31207. Should the manuscript be accepted, the data supporting the results will be archived in an appropriate public repository (Dryad, Figshare or Hal).

Abstract

Understanding whether land use intensification causes regime shifts is of key importance for management, particularly if these shifts are associated with thresholds separating different ecosystem states and with hysteretic dynamics. Here we use a unique, long-term grassland database to identify thresholds in the response of 16 ecosystem functions and the diversities of 21 taxa to land use intensity. We show that aboveground diversity (5 of 10 taxa), shoot biomass and soil N retention showed threshold responses to land use intensity, i.e., abrupt changes between extensively and intensively managed grasslands. Time-series analysis revealed that ecosystem functions showed hysteresis around the threshold, while diversity did not. Shifting back to the functioning seen in extensively managed grasslands may therefore require larger reductions in land use intensity than shifting to the high intensity state. Identifying such thresholds along land use gradients is critical to prevent ecosystem degradation and conserve biodiversity and ecosystem functions.

Introduction

Land use intensification is a major cause of biodiversity loss across trophic levels (Allan *et al.* 2015; Rounsevell *et al.* 2018) and leads to a homogenization of community composition (Gámez-Virués *et al.* 2015; Gossner *et al.* 2016). In addition, increasing land use intensity decreases ecosystem multifunctionality by increasing yield at the expense of other ecosystem functions (*e.g.* soil C storage and nutrient retention) (Soussana & Lemaire 2014) and services (*e.g.* cultural and aesthetic value, pollination and pest control) (Allan *et al.* 2015; Dainese *et al.* 2019). Land use effects on ecosystems are frequently non-linear, which translates into abrupt changes in grassland biodiversity and functioning as land use intensity increases (Kleijn *et al.* 2009; Allan *et al.* 2014). However, few studies have tested whether these non-linear changes result in regime shifts, *i.e.* persistent, large changes in system-state variables, such as biodiversity or ecosystem functions (Scheffer & Carpenter 2003). Identifying regime shifts is therefore central for determining the sustainability of different forms of ecosystem management, particularly when they affect human well-being (Crépin *et al.* 2012). Thus, in order to prevent losses of services and to avoid ecosystem degradation it is critical to know whether land use intensification leads to regime shifts in grasslands.

When regime shifts occur abruptly, they can be associated with threshold responses where a small change in an external driver, *e.g.* land use; leads to sudden and large changes in system-state variables, which are further accelerated by internal feedbacks (Suding *et al.* 2004; Briske *et al.* 2006; Ratajczak *et al.* 2018). Theories postulate two types of dynamics associated with thresholds (Figure 1) (Suding & Hobbs 2009): in

the first case, the system can transition back to its previous state when the driver returns to its previous level, *i.e.* the regime shift is reversible. However, in the second case the system may not easily transition back to the previous state, that is hysteresis occurs and there is a critical transition among different stable states. With hysteresis, the transition between states cannot be simply reversed by restoring the driver to its previous level, it requires larger changes (Scheffer & Carpenter 2003; Dakos *et al.* 2019). Previous studies identified thresholds for grassland vegetation responses to grazing (Briske *et al.* 2005; Sasaki *et al.* 2008), but none have tested for thresholds in the response of multiple aspects of diversity and ecosystem functioning to several elements of land use, or whether thresholds are associated with different ecosystem states and hysteresis.

Although threshold responses have sometimes been observed (Sasaki *et al.* 2015), several studies have suggested that there is no quantitative evidence for them and that thresholds are rarely detectable (Hillebrand *et al.* 2020). Several factors may explain these mixed findings: firstly, different ecosystem functions and the diversities of different taxa, are likely to vary in their response to external drivers (Allan *et al.* 2015; Newbold *et al.* 2015; Soliveres *et al.* 2016), and the absence of a threshold for one function or diversity does not preclude the existence of thresholds for others. However, most studies looking for thresholds focus on only a small number of variables. In general, studies on thresholds have focused on diversity measures and have rarely considered ecosystem functions (Sasaki *et al.* 2015) (but see (Evans *et al.* 2017)). Secondly, consistently detecting thresholds in response to external drivers can be challenging when ecosystem responses are highly variable (Hillebrand *et al.* 2020), meaning that spatially extensive datasets are needed to robustly identify thresholds. Finally, extensive sampling needs to be combined with long-term data to identify critical transitions in ecosystem states, *i.e.* using early warning signals (indicators of a system approaching a critical transition (Scheffer *et al.* 2009)), or to evaluate the dynamics associated with thresholds, *i.e.* to test for hysteresis (Sasaki *et al.* 2015), and such data are rarely available. Thus, testing for thresholds in ecosystem responses requires studies with continuous measures of external drivers and ecosystem responses across many sites and multiple years.

In this study, we use a large database from 150 temperate grasslands to identify thresholds in the response of the diversity of 21 taxonomic groups (including plants, arthropods, birds, bats and soil microbes), and 16 ecosystem functions (including productivity, measures of nitrogen, carbon and phosphorus cycling and herbivory and pathogen attack), to a gradient of land use intensity. We considered effects of land use components, grazing, mowing and fertilization, both individually and combined in a composite land-use index (Blüthgen *et al.* 2012). We asked the following questions: (i) do diversities of multiple taxa (across multiple trophic levels) and ecosystem functions show a threshold response to land use? and if so, (ii) are these thresholds indicative of critical transitions and hysteresis? To answer these questions, we used a three-step analysis. First, we identified which variables showed a non-linear response to land use intensity and tested whether those responses were associated with specific thresholds. Second, we looked for early warning signals of critical transitions, by testing for increased spatial variation along the land use gradient. Finally, we used 12 years of land use data to identify hysteretic dynamics, based on whether observed thresholds changed depending on the land use history (*i.e.* if land use intensity had recently decreased or increased on a grassland).

Methods

Study area

The studied grasslands are part of the Biodiversity Exploratories project (www.biodiversity-exploratories.de) (Fischer *et al.* 2010) and are located in three different regions of Germany: the Schwäbische Alb plateau, as a part of the UNESCO Biosphere Reserve Schwäbische Alb (south-west), Hainich-Dün region (central) and the UNESCO Biosphere Reserve Schorfheide-Chorin (north-east). The regions differ in geology, topography, climate and soils (Fischer *et al.* 2010). Detailed information about the studied regions can be found in Supplementary Table S1. In 2007, 50 permanent grassland plots of 50 m x 50 m were established in each region (150 in total). Plots had been grasslands for at least 20 years before the start of the project.

Land use intensity

Plots in all three regions were selected to cover land use gradients typical for central Europe (Blüthgen *et al.* 2012). To assess land use intensity (LUI), annual questionnaires were sent to landowners asking them about the intensities of grazing, mowing and fertilization on each plot (Blüthgen *et al.* 2012; Vogt *et al.* 2019). For grazing intensity (G), farmers reported the type (cattle, horse and sheep), stocking density and the duration of grazing periods (standardized to livestock units \cdot grazing days \cdot ha⁻¹ \cdot year⁻¹). For mowing frequency (M), farmers reported the number of annual cuts (number of cuts \cdot year⁻¹), and for fertilization (F), they reported the total fertilizer addition from which we calculated the amount of nitrogen added (kg N \cdot ha⁻¹ \cdot year⁻¹). The individual components (G, M, F) were then standardized to their means across regions, and a continuous compound index of LUI was calculated by summing the standardized components (LUI = G + M + F) (Blüthgen *et al.* 2012).

Biodiversity

Taxonomic diversity data were collected for 21 above and belowground taxa. Aboveground diversity was measured for vascular plants, lichens, bryophytes, arthropods (divided into herbivores, omnivores, predators, detritivores and pollinators) and vertebrates (granivores and insectivores). Belowground diversity included arthropods (divided in predators and detritivores), bacteria, fungi (divided into decomposers, pathogens, symbionts and others) and protists (divided into bacterivores, eukaryvores, omnivores and parasites). Species diversity for vascular plants, bryophytes and lichens was collected in a 4 m x 4 m subplot located in the middle of each plot. Arthropod species diversity was collected aboveground, using sweep netting along 150-m transects, and belowground, using soil core extractions. Bird species diversity was collected using standardized 5-minute point-counts, recording all individuals seen and heard from the center of the plot. Bat species diversity was measured using standardized acoustic monitoring. Diversity of bacteria, fungi and protists was assessed as the number of operational taxonomic units (OTU), based on metabarcoding approaches using DNA extracted from plants and soil. We defined grassland taxonomic diversity for each group as the number of species/OTUs identified. Detailed information about the collection of diversity data can be found in Supplementary Table S2.

Ecosystem functions

A total of 16 ecosystem functions were measured on each plot. Function data included grassland productivity (above- and belowground), proxies of energy transfer between trophic levels (herbivory, seed and dung depletion, litter decomposition, root decomposition, pathogen infection), biogeochemical cycling (carbon, nitrate and ammonia fluxes, phosphatase, potential nitrification, N and P retention), and hydrology (water recharge). Plant shoot biomass was measured in eight 0.5 m x 0.5 m squares and root biomass in 14 soil cores per plot. Herbivory was calculated by measuring the proportion of leaf area damaged by insects, on 100 random leaves selected from the biomass sample. Seed and dung depletion was calculated as the proportion of cattle dung or sunflower seeds removed after 48 hours exposure. Litter and root decomposition was calculated as the mass loss from litterbags after 4 and 6 months respectively. Pathogen infection was estimated by visual inspection and calculated as the cover weighted mean of pathogen prevalence and severity per plant species. Proxies of carbon, nitrate and ammonia fluxes were calculated using composite indices including several enzymatic activities (Carbon: β -Glucosidase, N-Acetyl- β -Glucosaminidase and Xylosidase; Nitrate: Denitrification enzyme; Ammonia: Urease) and functional genes (Nitrate: nitrogenase, nrxA gene for *nitrobacter*, 16S rRNA gene for *nitrospira*; Ammonia: ammonia oxidation genes for archaea and bacteria). C-cycling enzyme activities were analyzed using fluorogenic substrate and fluorometric detection, N-cycling enzyme activities as well as potential nitrification were photometrically analyzed and the abundance of functional genes was quantified via real-time qPCR analysis. Nutrient retention (N, P) was measured as the remaining anion content in resin bags buried 20 cm deep for 140 days. Water recharge was calculated using a water balance model using climatic and soil data. Detailed information about function data collection is included in Supplementary Table S3).

Statistical Analyses

Identification of thresholds in the response of diversity and function to land use

Before testing for thresholds, we checked whether any variables showed non-linear responses to land use intensity and its three components. To detect non-linear responses, we compared three models: linear regressions, quadratic regressions, and generalized additive models (GAM) (Hastie & Tibshirani 1990). Quadratic models allow for non-linear effects, with one turning point in the relationship between the variables, while GAMs allow for complex non-linear responses, with several turning points. Both quadratic models and GAMs are often used to identify ecological thresholds (Berdugo *et al.* 2020). The best fitting model for each response was selected using the Akaike Information Criterion. Specifically, we considered that quadratic models and GAMs had better fits than the linear model when they had an AIC values at least 2 units lower than the linear model ($\Delta AIC = AIC_{lin} - AIC_{qua,GAM} > 2$) (Burnham & Anderson 2003). Then, for variables that showed a nonlinear response, we tested whether they also had a threshold (*i.e.* an abrupt shift) in their response to LUI, and its three components, using segmented regression (Muggeo 2003). Segmented regressions were only applied to those variables that showed non-linear responses because they force break points in the explanatory variable, potentially leading to overfitting and spurious thresholds, if the response is linear. In addition, if GAMs suggested the existence of several turning points, we fitted segmented regressions with more than one break point. When the segmented regression model was significant and indicated a better fit than the linear model (based on $\Delta AIC > 2$), we considered that the variable showed a threshold response. To control for the effect of region on diversities and functions, we used the residuals of each variable from a model including region as the explanatory variable. All variables were standardized by subtracting the mean and dividing by the standard deviation before running the models. We used the *gam* (Hastie 2019) and *segmented* (Muggeo 2008) packages in R.

As land use intensity varied over time in each plot we always tested the response of each diversity and function to the land use intensity the previous year (*e.g.* the response of plant diversity in 2013 to grazing in 2012). Similarly, when variables were measured in several years, the mean value for the time period was used for both explanatory and response variables (*e.g.* the response of average plant diversity during 2011-2013 to average grazing during 2010-2012). We decided to use the land use value from the previous year because the study area is continuously managed and the different taxa and ecosystem functions may be measured before much of the land use in a given year has occurred. However, although LUI changes over time, the changes are not sufficient to dramatically alter the land use levels of the plots: both LUI and its components showed strong positive correlations between years (mean $r \pm$ standard error; LUI $r = 0.7 \pm 0.01$; grazing $r = 0.65 \pm 0.01$; mowing $r = 0.74 \pm 0.01$; fertilization $r = 0.71 \pm 0.01$) and all years were strongly related ($r > 0.74$) to the mean values over the total time period (Supplementary Figure S4).

Test of early warning signals at ecological thresholds

To identify early warning signals of critical transitions, we evaluated the variability of the diversity and function measures along the land use gradients. An increase in variability has been considered an early warning signal for regime shifts between alternative states (Scheffer *et al.* 2009). To test for this, we used a moving window approach along the land use gradients. All plots were sorted from lowest to highest LUI, or its components, and then, the variance of each diversity and function measure was calculated across each subset of 15 plots along the gradient (*i.e.* if plots are ranked from 1 to 150, the first subset included plots 1-15, next subset plots 2-16, until the last subset included plots 136-150). Each subset accounts for 10% of the plots and covers a range of 0.2 ± 0.01 LUI, 26 ± 1.3 livestock unit \cdot grazing days \cdot ha⁻¹ \cdot y⁻¹, 0.25 ± 0.01 cuts \cdot y⁻¹ and 6.4 ± 0.6 kg N \cdot ha⁻¹ \cdot y⁻¹. To test if the variance in a given subset was significantly different from expectation, the expected variance for any subset was estimated by assembling 2000 subsets of 15 random plots and estimating the variance for each random subset. We then calculated the 95% confidence interval across these random subsets as the values at percentiles 2.5 and 97.5. Although early warning signals were originally developed for temporal data, spatial gradients are frequently used as proxies, when appropriate temporal series are not available (Blois *et al.* 2013; Kéfi *et al.* 2014; Eby *et al.* 2017). Nevertheless, to prevent potential confounding factors causing high variability related to local environmental conditions (Huston 1999), we tested for increasing variability through time for those variables with temporal data available. Specifically, we calculated for each plot, the variance through time of the functions or diversities. We then related the temporal variance in diversity or functioning to the average LUI of the plot. Results using temporal variation

were similar to those using spatial variation (higher variance at LUI values close to the threshold, Figure S5).

Evaluation of hysteretic responses in the ecosystem

Finally, we evaluated if ecosystem thresholds were indicative of hysteretic responses to land use, by determining whether responses were different when LUI and its components increased or decreased over time. If hysteretic responses occur, we would expect different trajectories of diversity and function change when LUI increases or decreases, and therefore a higher threshold along the LUI gradient when land use is intensified. Therefore, for each plot we determined whether LUI had increased from the previous year (land use intensification) or decreased from the previous year (land use extensification). All cases where land use did not change between years were excluded. We also did not consider mowing as there were not enough thresholds to test for hysteresis. We analyzed all variables for which we had identified a threshold response to LUI and repeated the threshold detection analysis to test for different thresholds when land use had recently increased or decreased. If hysteresis occurs, then we would expect lower thresholds if land use has recently decreased (extensification). Specifically, for each combination of variable and year we ran a separate analysis for those plots that had experienced intensification or extensification. We ran individual analyses for each year because plots could show periods of increasing or decreasing land use at different times (on average across years; LUI: intensification = 71.9 ± 3.3 plots, extensification = 71.5 ± 3.2 ; Grazing: intensification = 55 ± 2.9 plots, extensification = 54 ± 3 ; Fertilization: intensification = 30.6 ± 1.5 plots, extensification = 33.6 ± 1.4). Finally, we tested if the thresholds for plots undergoing intensification or extensification differed significantly, as an indicator of hysteretic dynamics. To do this, we fitted a linear mixed effect model using plot category (intensification and extensification) and variable type (diversity or function), together with their interaction, as explanatory fixed variables, threshold value as the response variable and year as a random factor. Linear mixed effect models were fitted with the *lme4* package in R (Bates *et al.* 2015). All statistical analysis were done with R 3.6.2 (R Development Core Team 2014).

Results

Identification of ecological thresholds

We found that almost a third (29.3%) of the diversities and ecosystem functions responded non-linearly to land use intensification (Table S4.1). We found more non-linear responses for diversities (9 of the 21 taxa) than for functions (2 of the 16 functions). In general, aboveground taxa showed more non-linear responses to land use (5 of the 10 taxa), than belowground taxa (2 fungal guilds out of the 11 taxa). For the 11 variables which showed non-linear responses to land use intensification, we identified 9 (diversity of 7 taxa and 2 functions, Figure 2) with a threshold response. Thresholds were remarkably consistent between variables and were around a land use intensity (LUI) value of 1 (most aboveground diversity metrics) and 1.5-2 (fungal diversity, plant shoot biomass and N retention). To better understand what this threshold means we calculated the mean value of individual components across the 24 plots with average LUI within the expected threshold value ($LUI = 1.42 \pm 0.12$): grazing = 130.9 ± 24.5 livestock units \cdot grazing days \cdot ha⁻¹ \cdot year⁻¹; mowing = 1 ± 0.2 cuts \cdot year⁻¹; and fertilization = 4.9 ± 1.6 kg N \cdot ha⁻¹ \cdot year⁻¹. The transition threshold is therefore associated with a switch from lightly grazed, unfertilized grasslands, to fertilized, and frequently mown or grazed grasslands.

We also found that diversities of several trophic levels and functions had threshold responses to each land use component (Figure S1.1-3). Most of these thresholds occurred at very low levels, which probably represents a threshold between the presence or absence of any land use component (31% of grasslands considered were not grazed, 35% were not mown and 60% were unfertilized).

Early warning signals at ecological thresholds

We found most variables showed an increase in variance at LUI values close to, or within, the thresholds, although only a third of them showed a significant increase in variance, relative to random expectations (3 of 9, vascular plants, lichens and vertebrates granivores, Figure 3). However, we also observed significant

increases in variance at a similar LUI values for some belowground variables that did not show a threshold response to land use intensity (*e.g.* Symbiotic fungi, Bacteria, Root biomass, Figure S2).

Hysteretic responses in the ecosystem

We found that thresholds for ecosystem functions occurred at higher LUI than for biodiversity (Figure 4). Additionally, these LUI thresholds observed for ecosystem functions occurred at lower land use levels in plots where LUI had decreased compared to the previous year (extensification) than in plots where LUI had increased (intensification, Figure 4, Figure S3). This indicates that the observed critical transition for functions in grasslands is hysteretic and returning to previous functioning levels requires lower land use intensity than is needed to change functioning as land use is intensified.

Discussion

Identification of ecological thresholds

Our analysis found that, in general, aboveground taxa were more likely to show threshold responses to land use intensity while belowground diversity and ecosystem functions had more linear responses. This agrees with findings showing that biodiversity is more likely to show a non-linear response to management (Evans *et al.* 2017), and often declines non-linearly with land use intensification (Kleijn *et al.* 2009; Allan *et al.* 2015). However, while land use intensification reduces aboveground diversity by homogenizing environmental conditions, by increasing arthropod mortality (grazing and mowing) or by favoring particular species (fertilization), belowground diversity may be increased by nutrients inputs to the soil (Chen & Wise 1999). In addition, the long management history of the studied grasslands may have reduced belowground responses to land use intensity, as many belowground communities may be affected more by soil history than recent changes in aboveground composition (Elgersma *et al.* 2011). Regarding functions, the lack of non-linear responses could be explained by ecological redundancy (Walker 1992). It is possible that the loss of functionally important species with land use intensification can be partially compensated for by the remaining species, thus reducing abrupt responses (Muradian 2001). These contrasting responses of biodiversity (above and belowground) and ecosystem functioning highlight the need for system level approaches to understand overall consequences of land use intensification.

Remarkably, all variables showed thresholds at similar land use intensity (LUI) values. This might suggest that plant communities undergo a regime shift due to land use intensification that cascades to other taxa and functions. We observed two types of grasslands: extensively managed (LUI < 1) grasslands, with high aboveground diversity (especially of primary producers and vertebrates) and high nutrient retention, which are lightly grazed and unfertilized and intensively managed (LUI > 1.5) productive grasslands with high belowground fungal diversity (Figure 2), which are fertilized and mown or intensively grazed. Low grazing intensities promote plant diversity by limiting dominant species (Maurer *et al.* 2006; Bochet *et al.* 2016; Busch *et al.* 2019), while the fertilizer addition associated with higher mowing frequencies selects for more competitive, taller and faster-growing plants at the expense of smaller and slower-growing ones (Gough *et al.* 2001; Dickson *et al.* 2014; DeMalach *et al.* 2017; Busch *et al.* 2019). In the case of vertebrates, intensification in grasslands reduces diversity by limiting nesting options and increasing the risk of nest discovery by predators (Verhulst *et al.* 2004). In addition, biodiversity losses can cascade between trophic groups, as a reduction in primary-producer diversity affects herbivores by reducing the diversity of resources available to them (Uchida & Ushimaru 2014). Fertilization and increased dominance of fast-growing species in intensive grasslands affects ecosystem functioning by increasing aboveground plant biomass (Lavorel & Grigulis 2012; Allan *et al.* 2015). However, this increase may be non-linear, as environmental factors (*e.g.* soil water levels and light) limit further increases in biomass (Kleinebecker *et al.* 2014), or because the diversity loss associated with high fertilizer inputs results in a reduced increase in biomass at the highest levels of fertilization (Isbell *et al.* 2013a). The increase in aboveground plant biomass can also affect fungal communities (Voříšková & Baldrian 2013), as more organic matter may increase fungal decomposer diversity (Cline *et al.* 2018). In turn, N retention decreases in highly intensified grassland soils as a result of higher N inputs and the dominance of fast-growing species with low root density (Ledgard *et al.* 2011; Kleinebecker *et al.* 2014). Our findings

show that these well-known changes due to land use intensification may occur abruptly once a key threshold is crossed, which highlights the need to maintain low levels of land use to prevent rapid declines in diversity.

Thresholds associated with anthropogenic disturbance have been described for some ecosystems, such as eutrophicated lakes, and overgrazed drylands (Suding *et al.* 2004), but there had been little evidence for their importance in temperate grasslands (Sasaki *et al.* 2015). Some previous studies have found abrupt changes in certain ecosystem-state variables in response to particular land use components (*e.g.* increasing grazing intensity changed vegetation composition; fertilization changed vegetation composition and soil properties) (Suding *et al.* 2005; Sasaki *et al.* 2008; Ramirez *et al.* 2010). In our analysis most changes in response to individual components were associated with the presence or absence of the component. It is well known that grazed and ungrazed or fertilized and unfertilized grasslands differ dramatically (Bai *et al.* 2010, 2012). However, our results also show thresholds in response to an integrated measure of land use intensity, suggesting that it is the combined effect of changes in multiple land use components that causes the abrupt changes in ecosystem states. Global change drivers usually operate synergistically by changing ecosystem feedbacks and leading to regime shifts that would not have happened if drivers acted individually (Suding *et al.* 2004; Briske *et al.* 2005; Rillig *et al.* 2019). Altogether, our results confirm the large impact that land use intensification has on ecosystems (Thébault *et al.* 2014; Newbold *et al.* 2015, 2016), while providing novel evidence for the existence of regime shifts in the diversity of aboveground primary producers and vertebrates, biomass production and nutrient retention in managed temperate grasslands.

Early warning signals at ecological thresholds

Early warning signals, such as increasing variability near a threshold, have been suggested as indicators of a system being about to switch between stable states (Scheffer & Carpenter 2003; Scheffer *et al.* 2009; Kéfi *et al.* 2014). Finding evidence for an increase in variance would therefore provide an indication that the thresholds we detected are associated with critical transitions. We found significant increases in variance for plants and some birds, suggesting they may experience a critical transition. However, several belowground variables also showed significant increases in variance but not a threshold response, which suggests that an increase in variance may not be a reliable early warning signal. Alternatively, it is possible that the abrupt transition in the plant community has cascading effects on other processes and changes their variance but not mean values. In grasslands, nutrient addition causes a shift from slow growing conservative, to fast growing acquisitive plant species, which is associated with increased productivity and faster soil nutrient cycling (Eskelinen *et al.* 2020). At the transition between these two states there may be greater variability in plant functional composition between grasslands, which results in greater variability in various belowground processes. The existence of a critical transition between plant communities in managed grasslands would have important consequences. Extensively and intensively managed grasslands provide different ecosystem services, and a critical transition between these states would suggest that land use intensity needs to be kept well below the threshold in order to preserve the extensively managed, high-diversity state.

Hysteretic responses in the ecosystem

Our results showed that LUI thresholds for ecosystem functions can depend on whether land use recently increased or decreased, suggesting hysteretic dynamics. This hysteretic response indicates that returning to previous functioning levels requires lower land use intensity than is needed to change functioning as land use is intensified. Previous studies have suggested hysteretic dynamics in grasslands due to nutrient enrichment after fertilization (Isbell *et al.* 2013b), as nutrients can persist in the soil for many years (Hrevušová *et al.* 2009; Spohn *et al.* 2016). Thus, the slower recovery of nutrient retention, and slower decline in plant productivity, as land use is extensified may occur because fast-growing plants can maintain high dominance and productivity for some years after land use is reduced (Baeten *et al.* 2011). On the other hand, diversity thresholds were not affected by the direction of change in land use, suggesting a lack of hysteresis (Figure 4). This may be because diversity responds more slowly (Bommarco *et al.* 2014; Löffler *et al.* 2020). We only looked at whether land use intensity had increased or decreased relative to the previous year; however, if diversity responds more slowly than this, then only the long-term mean land use intensity on a plot may have an effect. We were not able to look at longer-term changes in land use as only 4% of plots experienced

decreased, and 2% of plots increased, LUI for more than 5 consecutive years. Thus, although some studies have suggested that limited intensification can produce optimal biodiversity and functioning in grasslands (Yang *et al.* 2018), we do not find evidence for this. In our study area land use extensification (*i.e.* reducing fertilization while keeping high grazing or mowing levels) will not immediately reduce yield but it will also not support a rapid recovery of diversity. Our results therefore suggest that LUI needs to drop below 1 before high diversity grasslands could be promoted, *i.e.*, no fertilization and low grazing or mowing. Finally, as abandonment of grassland management will lead to natural succession and the replacement of these semi-natural grasslands by shrubland and forests, extensive land practices are essential to preserve diverse grasslands (Queiroz *et al.* 2014). The existence of hysteresis associated with land use has key implications for conservation strategies, which further reinforces the idea that land use intensity needs to remain well below the threshold to preserve biodiversity.

Conclusions

We found evidence for thresholds in how land use intensification affects aboveground diversity and for a transition from extensively managed grasslands with high aboveground diversity and high soil nutrient retention, to intensively managed grasslands with high biomass production and belowground fungal diversity, but low aboveground diversity. Identifying these thresholds is key to prevent abrupt declines of biodiversity (LUI should not increase above 1) and to find the optimal, efficient management level allowing high productivity with lowest inputs (a LUI close to 2). In addition, extensively managed grasslands with land use levels below the threshold are priority targets for nature conservation, as it may be more difficult to restore grasslands once the threshold is crossed. Our results highlight the importance of testing for complex effects of global change drivers on multiple ecosystem components across many sites and multiple years to account for the high variability in responses that limit our capacity to identify thresholds (Hillebrand *et al.* 2020). As global change drivers can lead to critical transitions, it is important to anticipate them in order to avoid undesirable changes in both diversity and ecosystem functioning.

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Figure 1. Trajectories for regime shifts in ecosystems . Ecosystems experience a regime shift when they show a large change in an ecosystem state (*i.e.* variables describing the ecosystem) (a). This change can be due to one or more environmental drivers and can be gradual or fast, leading to multiple relationships (unbroken black line) between ecosystem state and environmental drivers. The regime shift can be gradual if the ecosystem state changes linearly with the driver (b), or abrupt if the change shows a threshold (c). In addition, thresholds can show hysteresis if the direction of the change (grey arrow) is associated with different thresholds (d). In this case, bringing the driver back across the original threshold will not return the ecosystem to its previous state (broken black lines).

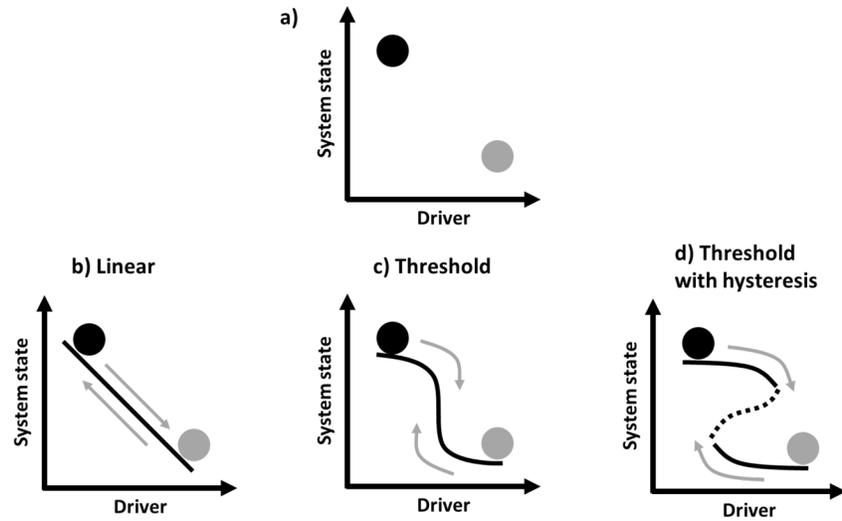


Figure 2. Threshold responses along LUI gradient for different taxa diversity and ecosystem functions. On the left, response to land use intensity (LUI) of all variables considered, including above- and belowground diversity, and ecosystem functions. Different colors indicate different responses to LUI (red/orange = negative response; blue = positive response; grey = no response; vascular plant diversity always decreased with land use intensity but the strength of that decline was lower after the threshold, which is indicated as a shift from red to orange). * indicates a variable showing a threshold response. On the right, one example of threshold response for above- (plants) and belowground diversity (fungal decomposers), and ecosystem function (N retention).

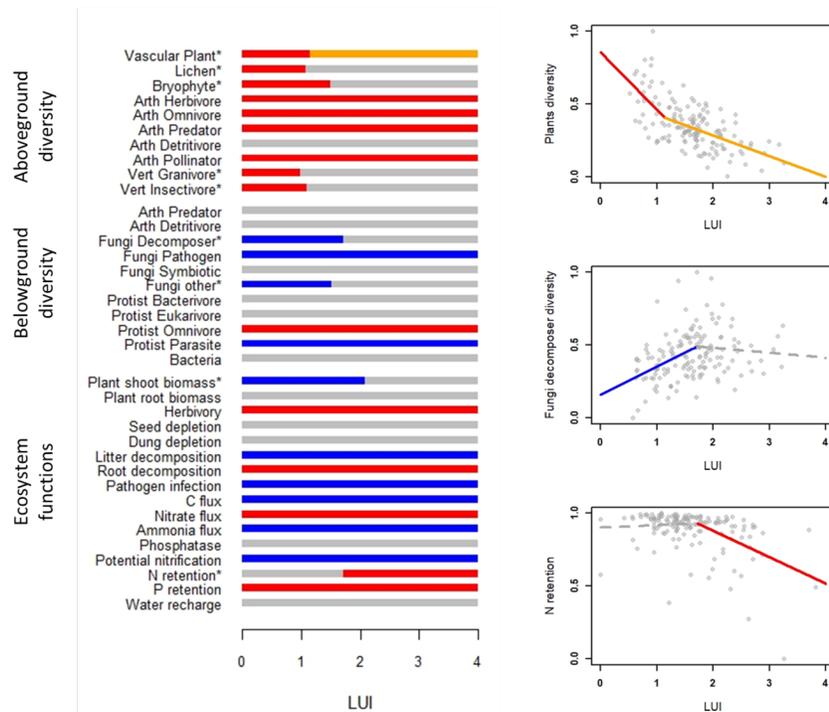
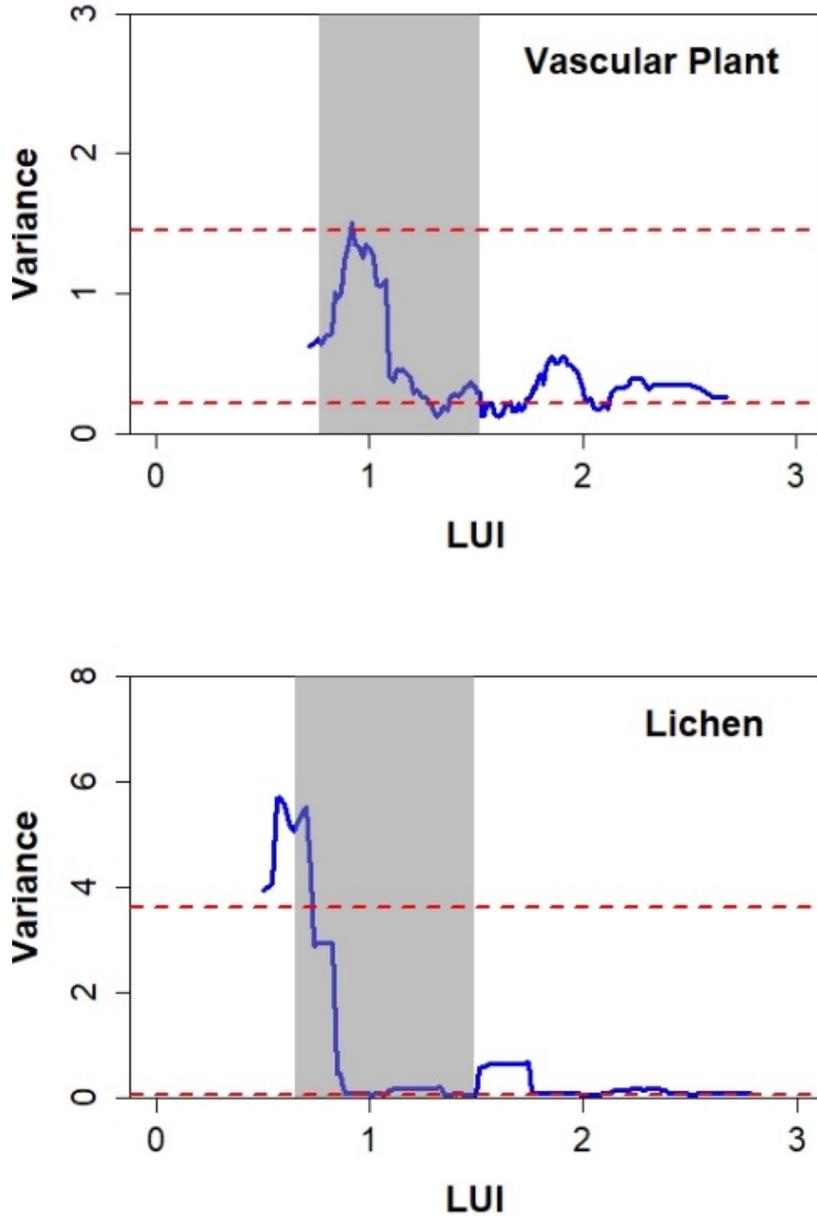
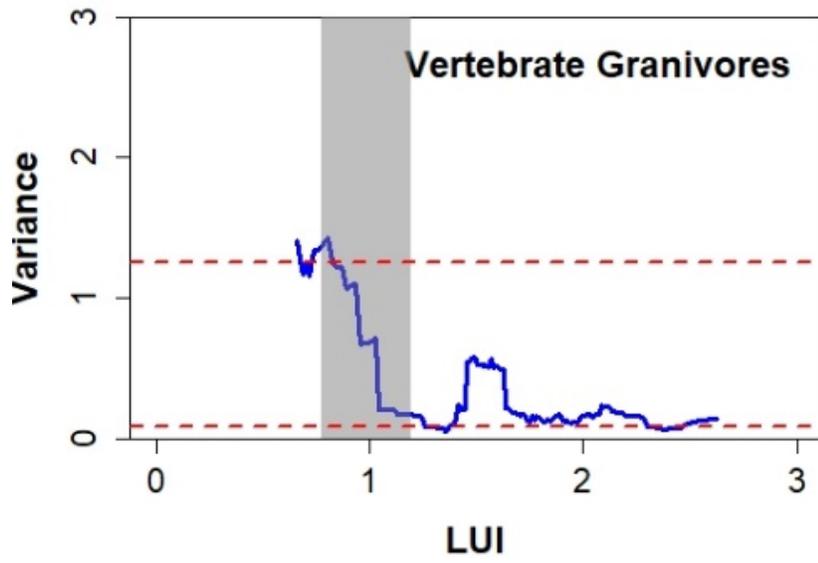
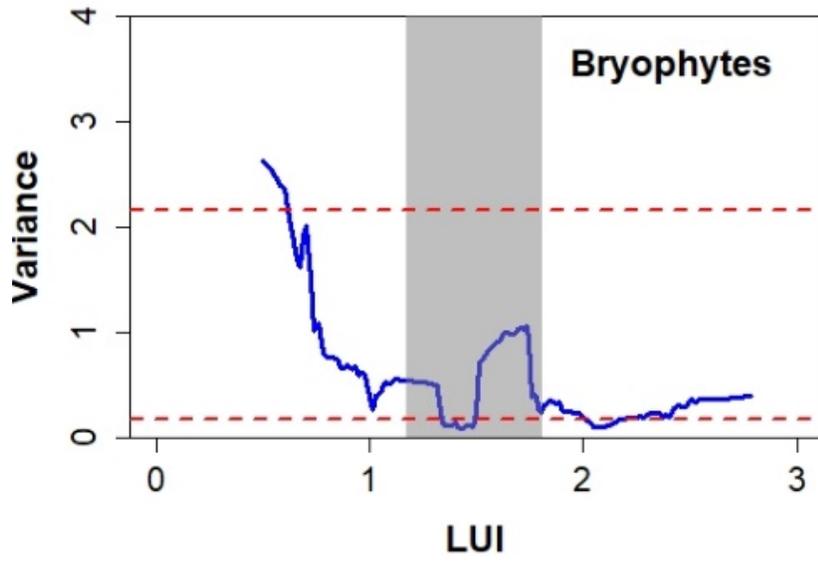
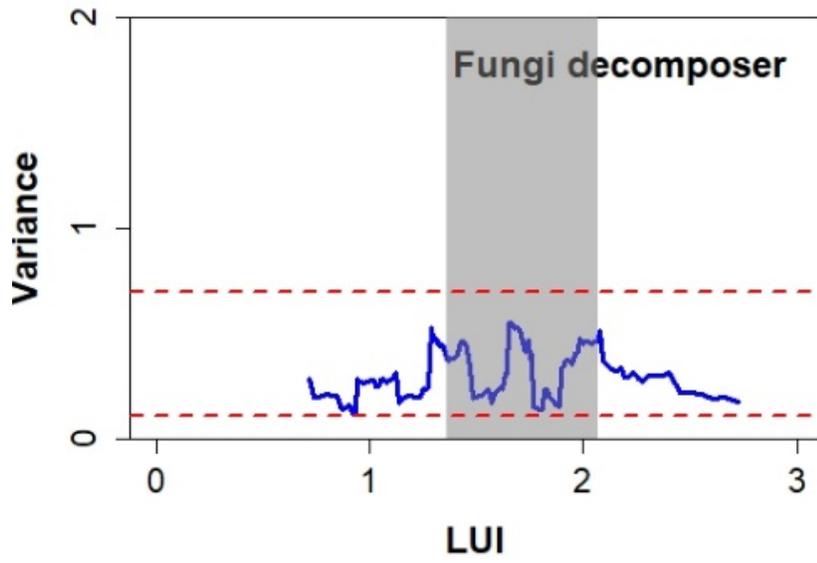
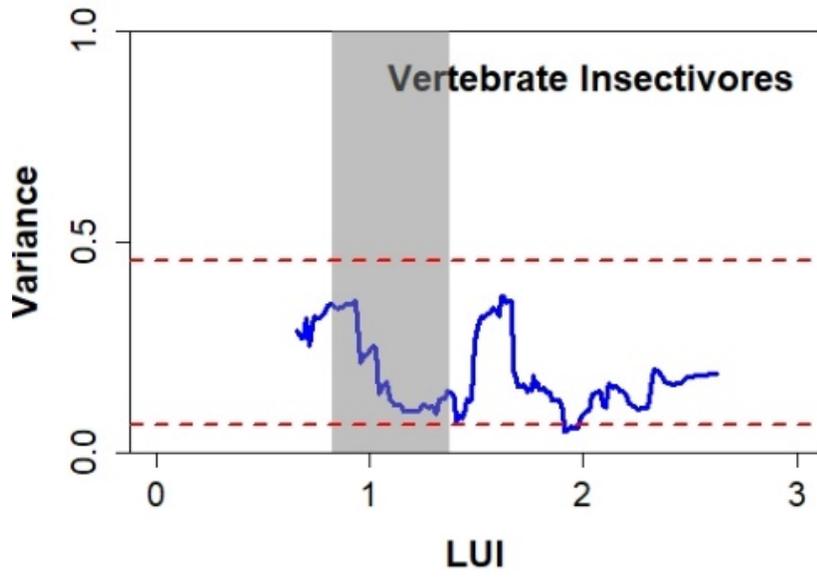
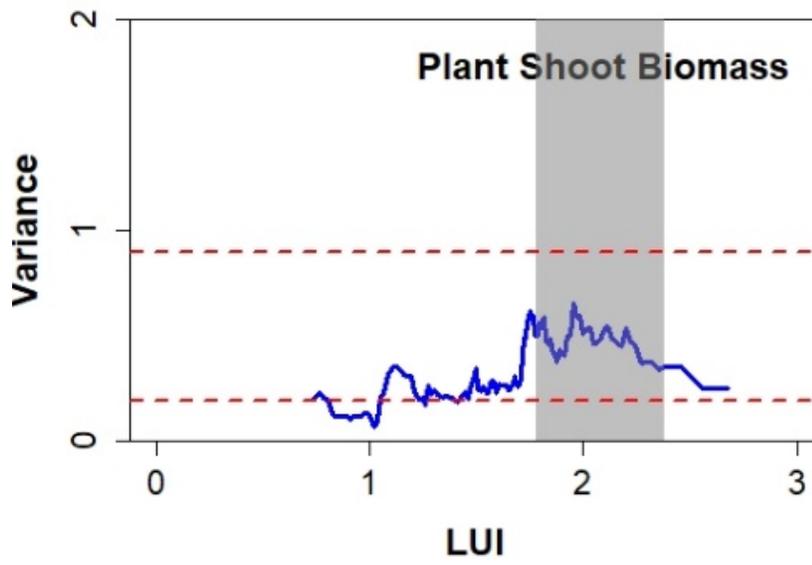
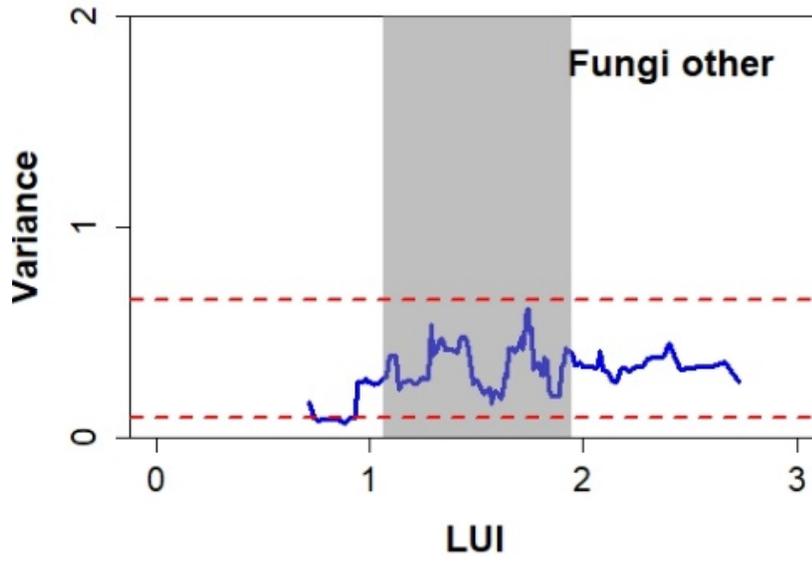


Figure 3. Variance for the considered taxa diversities and ecosystem functions showing a threshold response along the Land Use Intensity (LUI) gradient. The grey area represents the values along the gradient where a threshold was detected. Red dashed lines represent the confidence interval for the expected variance calculated based on bootstrapping the plots. Variance values higher than expected can be considered as indicative of a critical transition between ecosystem states.









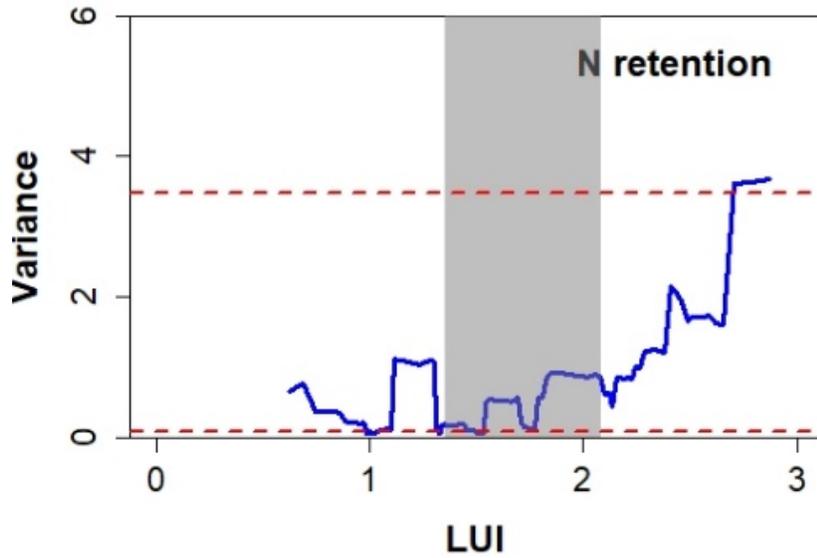


Figure 4. Variation in the Land Use Intensity (LUI) threshold depending on temporal changes in land use. Land use thresholds for diversities and ecosystem functions on plots where LUI had recently increased (intensification, blue line and dots) or decreased (extensification, orange line and dots). For diversity the threshold was not affected by the direction of recent changes in LUI but ecosystem functions had different thresholds depending on whether LUI had recently increased or decreased, suggesting hysteresis and the presence of a critical transition between two ecosystem states. Top left subplot shows the threshold response of plant diversity to LUI, which is the same for plots where LUI had increased or decreased compared to the previous year. Top right subplot shows hysteresis for the threshold response of plant shoot biomass, where the threshold is different on plots where LUI had increased compared to the previous year (blue) or where it had decreased (yellow). ** $p < 0.01$, *** $p < 0.05$.

