Historical and recent land use affects ecosystem functions in subtropical grasslands in Brazil

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Abstract. Land-use changes such as conversion of semi-natural grasslands to agriculture, silviculture, or high-intensity pastures affect biodiversity and ecosystem functions and services. However, which ecosystem functions are affected when highly diverse grasslands are converted remains largely unknown. As a model system, we studied 80 grasslands in Rio Grande do Sul, southern Brazil, comprising exceptionally diverse permanent grasslands that are traditionally managed with burning of accumulated biomass and moderate grazing, and four additional grassland types with different present or historical management: permanent grasslands with reduced or increased current management intensity and secondary grasslands after past agricultural or silvicultural use. We measured ten ecosystem functions covering all major below- and aboveground ecosystem components and the processes that link them, using the novel rapid ecosystem function assessment approach. Ecosystem functions included primary and secondary production, and species interactions, that is, herbivory, pollination, predation, seed dispersal, and decomposition. Ecosystem functions differed significantly among grassland types, most distinctly between permanent and secondary grasslands. Historical land-use changes to agriculture and silviculture led to altered ecosystem functions even after reconversion to grassland, including lower primary and secondary production, lower decomposition, lower seed dispersal capabilities, and higher invertebrate herbivory. Current management practices explained additional variation in some ecosystem functions, including strong positive effects of intensified management on secondary production. Other ecosystem functions such as pollination and predation were not affected. The findings suggest that conversion of grasslands to more intensive land-use types has long-lasting consequences for some ecosystem functions, with effects persisting even many years after reconversion, resulting in changes in the ecosystem services provided by these grasslands.

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INTRODUCTION

Ecosystem services that are essential for human well-being, such as clean water provisioning, soil erosion control, or the pollination of crops, depend on ecosystem functions that are controlled by the species living in an ecosystem (Millennium Ecosystem Assessment 2005). Human land use has altered a large portion of the earth's terrestrial surface (Ellis 2011) and is the main driver of biodiversity decline (Sala et al. 2000, Maxwell et al. 2016), thereby influencing the availability of these services (DeFries et al. 2004, Foley et al. 2005, Butchart et al. 2010, Lawler et al. 2014). Since the 1990s, many studies have assessed ecosystem functions as affected by biodiversity, often in controlled experiments. This line of research has generated ample evidence for positive effects of biodiversity on individual ecosystem functions and ecosystem multifunctionality (Balvanera et al. 2006, Cardinale et al. 2006, Soliveres et al. 2016). However, the transferability of findings from these highly controlled environments to real-world conditions has been questioned (e.g., Srivastava and Vellend 2005, Hillebrand and Matthiessen 2009). Additional interest in ecosystem functions comes from applied ecology, where ecosystem functions are increasingly used to evaluate restoration and conservation activities that aim at mitigating effects of habitat loss and land-use change (Meyer et al. 2015, Kollmann et al. 2016). Anthropogenic land use can impact numerous biotic and abiotic factors that directly or indirectly control ecosystem functions (Laliberté and Tylianakis 2012, Gossner et al. 2014), but we lack a more detailed understanding of the consequences of land use for ecosystem functioning. Many studies on land use focus on the effects of habitat conversion from natural ecosystems to anthropogenic landscapes like conversion of forests and grasslands to agricultural land or tree plantations (e.g., Dislich et al. 2017), analyze elements within anthropogenic landscapes (e.g., Smukler et al. 2010), or investigate effects of farming practices (e.g., Geiger et al. 2010). Fewer studies compare ecosystem functions among different levels of management intensity (e.g., Werling et al. 2014, Allan et al. 2015, Li et al. 2017), where effect sizes might expectedly be smaller than when assessing consequences of land conversion. As in biodiversity research, many studies on land-use effects have focused on single ecosystem functions (often on indicators of productivity) with fewer studies investigating multiple functions (e.g., Smukler et al. 2010, Allan et al. 2015, Li et al. 2017). Studying several ecosystem functions is necessary because individual ecosystem functions can differ and even contrast in their responses to changes in biodiversity (Allan et al. 2013) and land use (Allan et al. 2015). Consequently, there is a need to monitor multiple ecosystem functions after land conversion or restoration activities to improve our understanding of how ecosystem functions are affected by different land-use practices (Rey Benayas et al. 2009). This understanding is key for facilitating informed policy decisions (DeFries et al. 2004, Lemaire et al. 2011, Meyer et al. 2015).

A large variety of indicators for ecosystem functions and biodiversity has been used to investigate land-use effects (Feld et al. 2009). Applications range from studies solely considering soil-related functions (e.g., Maestre et al. 2012), to approaches focusing on functions that translate into relevant ecosystem services (e.g., Allan et al. 2015, Dislich et al. 2017), or on sets of varying "key" ecosystem functions (e.g., Werling et al. 2014). To enable a comprehensive assessment of ecosystem functioning, Meyer et al. (2015) proposed a rapid ecosystem function assessment (REFA) that measures indicators of ecosystem functions with easy-to-use and standardized methods. Applying the entire set of proposed REFA methods could provide a comprehensive overview of a terrestrial ecosystem. However, until now, the proposed methods have not been employed in a large-scale monitoring of ecosystem functioning.

Grasslands are among the ecosystems that are most strongly affected by human activity, with habitat conversion largely exceeding protection (Hoekstra et al. 2005). At the same time, grasslands cover approximately one-quarter of our

planet's terrestrial surface (Graetz 1994) and provide numerous ecosystem services (Sala and Paruelo 1997). In our study, the exceptionally species-rich grasslands of Brazil's southernmost state of Rio Grande do Sul served as a model for this biogeographical region. Grasslands in southeastern South America are increasingly experiencing fragmentation and reduction due to the expansion of cropland (Baldi and Paruelo 2008), as well as commercial afforestation with pine (Overbeck et al. 2007, Hermann et al. 2016) and eucalyptus (Jobbágy et al. 2006). For the grasslands in southern Brazil, also referred to as *Campos*, it is estimated that roughly half of the original grassland area had been replaced by 2002 (Cordeiro and Hasenack 2009). Only 2% of the grassland area is included in protected areas. As this protection in most cases implies the exclusion of land use of any kind (Brandão et al. 2007, Overbeck et al. 2007), that is, the exclusion of disturbances like fire or grazing, many areas are subject to diversity loss through competitive exclusion and the subsequent domination of tussock grasses, shrub encroachment, and transition to forests (Oliveira and Pillar 2004, Overbeck et al. 2005, Altesor et al. 2006). Therefore, even the protected grasslands may face degradation. More recently, demand for higher productivity has also led to the expansion of fertilized pastures that are overseeded with exotic forage species (Nabinger et al. 2009). In a recent study conducted in the *Campos* region, we have shown that abiotic conditions and plant species composition and richness were significantly altered by anthropogenic land-use changes (Koch et al. 2016). In short, conversion to other land-use types, intensified human land use, as well as the abandonment of management, threaten native grasslands in southern Brazil, with unknown consequences for ecosystem functioning and the resulting ecosystem services (Tornquist and Bayer 2009).

This study investigates on a total of 80 grassland plots previously used to assess land-use effects on vegetation composition (Koch et al. 2016), how multiple above- and belowground ecosystem functions vary among types of grassland in the highlands of the *Campos* region in southern Brazil with different levels of historical and recent management intensity. It analyzes how historical land uses, that is, agriculture, silviculture, and management as grassland, and current management practices, including burning or mowing, grazing, overseeding with exotic species and fertilizing, affect ecosystem functions. In the first large-scale application of the REFA methods proposed by Meyer et al. (2015), we quantified ten different ecosystem functions selected to cover all major ecosystem components and the often neglected processes that link them: primary productivity above (1) and below ground (2); secondary productivity above (3) and below ground (4); and several species interactions. The latter include invertebrate herbivory (5), which affects long-term plant species composition through altered competition and plant resource allocation (Karban et al. 1997, Weisser and Siemann 2004), arthropod predation (6), insect pollination (7), and seed dispersal (8), which influence plant community structure (Steffan-Dewenter and Tscharntke 1999). Additionally, we included decomposition (9) and the microbial biomass (10) in the soil, which are measures of the breakdown of organic litter and thus important for nutrient cycling and soil fertility. Based on our measurements, we addressed the following research questions:

1. Do ecosystem functions differ among traditionally managed permanent grasslands and four types of altered grassland ecosystems, that is, permanent grasslands with reduced or increased current management intensity and secondary grasslands after past agricultural or silvicultural use?
2. Are levels of ecosystem function in secondary grasslands that recover from a previous conversion to other land-use types different from those in sites with a continuous grassland history?
3. Do current management practices affect ecosystem functions?

Methods

Study region

Geography and land use.—The study was conducted in the highlands of Brazil's southernmost state Rio Grande do Sul, the so-called *Campos de Cima da Serra* region (Pillar and Lange 2015). Its grasslands are species-rich (Iganci et al. 2011) and considered to be a remnant of natural grasslands that dominated during the Early- and Mid-Holocene (Behling and Pillar 2007), shaped

by mammalian mega-herbivores and fire (Cione et al. 2003). The study area (Appendix S1: Fig. S1) is located between 28°58′ S and 29°26′ S, and between 49°58′ W and 50°36′ W, covering roughly 4800 km^2. In this region, the highlands reach 810–1125 m asl and mean annual temperatures are 16–22°C, with frequent frost in the winter months. Mean annual precipitation is 1500–1700 mm, without a marked dry season (Almeida 2009).

Five main grassland types occur in the *Campos* region, including three permanent and two secondary grassland types. All grassland types show distinct combinations of historical land use (managed as grasslands, agricultural fields, or pine plantations) with current management practices (burning or mowing, grazing, overseeding with exotic species combined with fertilizing; Table 1). The three permanent grassland types represent sites with an uninterrupted grassland history that only differ in their current management intensity: grasslands with medium, or traditional, management intensity, consisting of extensive grazing and burning every 1–2 yr (medium-intensity permanent grassland) and their high- and low-intensity variants. These are grasslands that undergo fertilization and overseeding with exotic forage species, high cattle stocking, and no burning (high-intensity permanent grassland), and grasslands that are excluded from any management intervention but do experience low levels of grazing (low-intensity permanent grassland). A minimum fire frequency of two events within the five years before

sampling was defined as a prerequisite for permanent grasslands with medium management intensity to avoid the inclusion of sites with only accidental or natural but rare fire events.

The two secondary grassland types undergo natural succession after a period of intensive use and are distinguished by the nature of their historical use, that is, sites that experienced agricultural use, including fertilization and tillage (secondary grassland after agriculture), and sites recovering from pine monocultures (secondary grassland after silviculture). Both secondary grasslands are stocked with cattle, with varying but usually high rates on former arable land, and lower rates on abandoned silvicultural land (Table 1).

Sampling site selection.—A total of 80 different sites with at least ten replicates of each grassland type were studied (Table 1). Each site was located within a connected area of homogeneous land use of at least 1 ha in size, with the additional condition of a medium- or low-intensity permanent grassland present within a radius of 2 km. Some sites were located in protected areas. Large protected areas potentially included more than one site, in which case the sites were separated by no less than 500 m. Suitable areas were chosen via review of satellite imagery, and central coordinates were randomly generated while guaranteeing a minimum buffer zone of 20 m from the edge to the nearest sampling point (Koch et al. 2016).

Land-use variables.—Information on historical and current land use was gathered through

Table 1. Studied grassland types and their historical and current management features and number of replicates.

Characteristic	Permanent grasslands			Secondary grasslands	
	Low intensity	Medium intensity	High intensity	After agriculture	After silviculture
Historical land use	Grassland	Grassland	Grassland	Agriculture	Silviculture
Years of non-grassland use	–	–	–	6.0 ± 1.1	23.8 ± 2.8
Years since land-use change	12.3 ± 1.5	–	11.2 ± 1.7	3.0 ± 0.5	4.3 ± 0.9
Grazing intensity	5.1 ± 1.3	6.1 ± 0.4	15.6 ± 3.9	13.8 ± 3.0	2.4 ± 0.3
Burning frequency	<2 in last 5 yr	≥2 in last 5 yr	0	0	0
Mowing	3 sites	2 sites	1 site	1 site	0 sites
Tillage	Never	Never	Never	During arable use	Never
Fertilization	Never	Never	Regularly	During arable use	Never
Overseeding with exotics	Never	Never	Regularly	During arable use and at abandonment	Never
No. sites sampled	19	17	10	19	15

Notes: Grazing intensity is measured in heads of cattle months, per ha and year. Years since land-use change indicates the time since the abandonment of agriculture or silviculture for secondary grasslands, and time since management cessation or intensifications for permanent grasslands. Given are descriptions for categorical variables and means with standard errors for numerical variables.

personal interviews with land owners, which is the most reliable source of information regarding past land-use changes and current management measures. Communication was direct and conducted in Portuguese. Each landowner was asked to provide information on the same standardized set of variables. For the secondary grasslands, the year of conversion from grassland to pine plantation or arable land and the year of abandonment of said use were recorded. For high-intensity and low-intensity permanent grasslands, the year of management intensity increase or decrease was noted. Furthermore, information on fire regime and grazing intensity in heads of cattle months per year and hectare was collected. Satellite imagery by Google Earth provided an additional source of information.

Measuring ecosystem functions

Sampling scheme.—Field work was conducted in two separate periods, the first from November 2013 to February 2014 (sites 1–40) and the second from November 2014 to January 2015 (41–80). The sampling periods during the summer months of the Southern Hemisphere ensured comparable levels of peak standing biomass and peak invertebrate activity. To avoid a seasonal or interannual bias, we sampled grassland types in random order. For measurements of ecosystem functions on each site, we employed a sampling scheme oriented along a north–south- and east–west axis, with the center coordinate at the axis' intersection (Fig. 1). This center point and four more points that were located at exactly 30 m from the center in each of the four compass directions constituted the northwest corners of five 5 m × 5 m multiplots (MPs). Inside these MPs, assessments of primary and secondary productivity, microbial biomass, and decomposition were conducted (Fig. 1). On three out of four randomly selected diagonals connecting the outer MPs, a process sampling point (PSP) was installed. At each PSP, disturbance-sensitive ecosystem functions, that is,
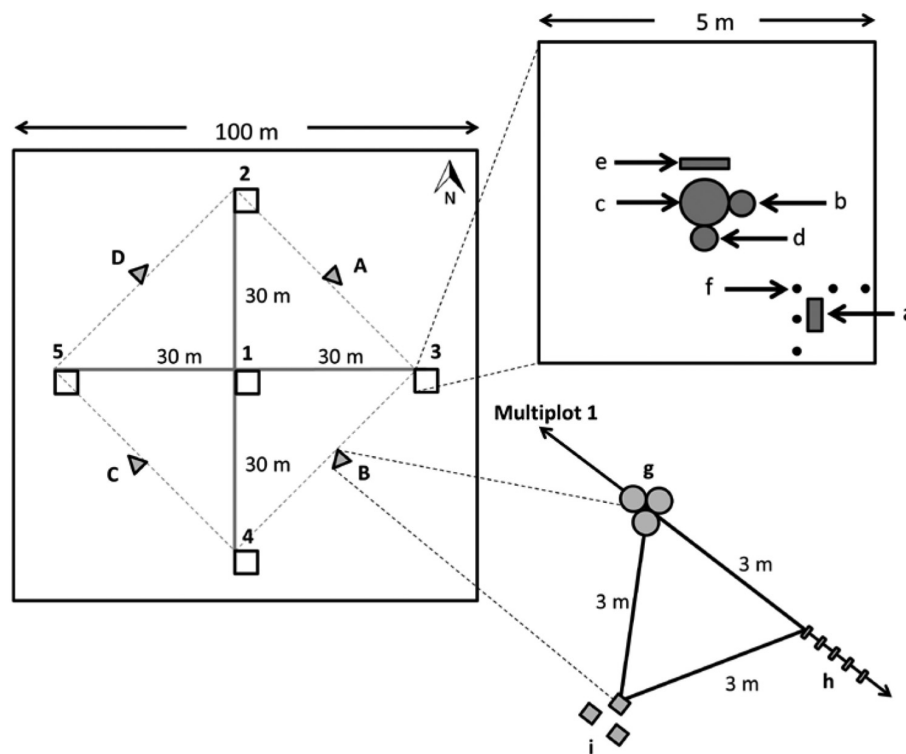

Fig. 1. Site sampling scheme with multiplots (1–5) and process sampling points (A–D); multiplot detail with sampling locations of standing biomass (a), root biomass (b), suction samples (c), belowground invertebrates (d), decomposition (e), and microbial biomass (f); process sampling point detail with sampling locations of pollinators (g), predation (h), and seed removal (i).

predation, pollination, and seed removal experiments, were assessed (Fig. 1).

Ecosystem function assessment.—We aimed at quantifying a broad range of ecosystem functions, with a special emphasis on functions mediated by species interactions involving invertebrates and plants. We used the REFA introduced by Meyer et al. (2015) to measure proxies of the ecosystem functions described below, following detailed field protocols described by Meyer et al. (2017). Sampling started at 9 am and lasted 8 h. Sampling duration was shorter when it started raining, but never <4 h.

Aboveground primary productivity.—Net primary productivity is defined as the total mass gain from photoautotrophic carbon fixation per unit ground area and time, after accounting for losses due to death, herbivory, exudation, or volatilization (Scurlock et al. 2002). Primary productivity was approximated by peak standing biomass in 20 cm × 50 cm plots in all five MPs (Fig. 1). Biomass was cut at ground level, including dead biomass and woody components of vegetation. Samples were dried for 48 h or until dry at 70°C before weighing.

Belowground primary productivity.—Standing root biomass, comprised of root biomass including root litter, was evaluated as an indicator of belowground net primary productivity (Ni 2004, Ravenek et al. 2014). Following Böhm (1979), volumetric soil samples of 10 cm depth and a diameter of 20 cm were taken with an auger in MPs 1, 2, and 4 (Fig. 1). Roots were separated by washing the soil sample through a sieve, and then dried for 48 h at 70°C before weighing; large woody pine roots were excluded from the analysis.

Aboveground secondary productivity.—Net secondary productivity is the rate of conversion of plant organic matter into heterotrophic tissues (Schowalter 2006). The abundance and fresh weight of invertebrate consumers served as an approximation of the magnitude of the consumer production. Invertebrates were sampled in MPs 1, 2, and 4 (Fig. 1) using suction sampling (Brook et al. 2008): 0.25 m^2 of vegetation was covered with a fine-meshed gauze cage to prevent arthropods from escaping and then sampled with a D-Vac (Stihl SH 86), equipped with a fine filter, twice for 1.5 min with a 30-s break. Sampling took place between 11 am and 1 pm at all sites to avoid bias due to daytime. Samples were transferred to 70% ethanol in the field and separated from plant material in the laboratory before being counted. Fresh weight was measured after removing excess liquid with tissue paper (Gehaka AG200; max = 201 g, d = 0.1 mg, e = 1 mg).

Belowground secondary productivity.—Abundance and fresh weight of invertebrates were used to approximate the magnitude of belowground consumer production. Large soil cores with a diameter of 20 cm and a depth of 10 cm were taken in MPs 1, 2, and 4 (Fig. 1). The soil fauna was extracted from the soil cores over a period of 10 d using an adapted Berlese-Tullgren-Funnel with a 30-W light bulb and collected in ethylene glycol (Southwood 1978, Edwards 1991). After sample cleaning, the number of individuals was counted and the fresh weight measured as described above.

Pollination.—Insect pollination was approximated using pan traps to assess pollinator abundance and fresh weight. Three pans of colors blue, yellow, and white (Nuttman et al. 2011), filled with water and a drop of detergent, were mounted at vegetation height at each PSP (Fig. 1). Samples from the three pans per PSP were pooled, and abundance and fresh weight of invertebrates were recorded as above.

Herbivory.—The amount of plant material consumed by animals links the producer and consumer pools (Schowalter 2006). The herbivory assessment was restricted to damage caused by invertebrates. Contrary to invertebrate herbivory, grazing is a component of a site's management and is therefore included in our analyses as such. Invertebrate herbivory is a measure of the ecosystem function that is not directly controlled by human management. Herbivory was quantified by calculating the proportion of damaged leaves following Souza et al. (2013). A total of 100 leaves were randomly drawn from vegetation samples of MPs 1, 2, and 4 (Fig. 1). Leaves from shrubs, forbs, and grasses were sampled proportional to their estimated relative biomass.

Invertebrate predation.—Potential predation on insects was assessed using green, cylindrical plasticine (Staedtler, Nürnberg, Germany) dummies of 20 mm length and 6 mm diameter (Howe et al. 2009, Low et al. 2014). Three (sites 1–40) or 10 (41–80) dummies were exposed at each PSP by pinning them to the ground on a 3 cm × 3 cm earth-colored paper in transects

spaced by 1 m (Fig. 1). Feeding marks on dummies were quantified and attributed to either arthropod, bird, or mammal predators (Low et al. 2014).

Seed dispersal.—The potential for seed dispersal was approximated with seed removal experiments. A standardized number of 25 halved sunflower seeds were placed on a plastic well plate with a regular grid of 5 × 5 positions spaced by 2 cm. Three such plates were exposed at each PSP, at a distance of 1 m to each other (Fig. 1). The number of seeds remaining on the plate was monitored at intervals of no more than 90 min. Removal probabilities indicating the likelihood of a single seed to be removed within 60 min were calculated with the survival package in R (Therneau 2015) assuming constant removal hazards.

Decomposition.—To assess decomposition rates, the weight loss of wooden sticks was measured (Reed et al. 2005). One (sites 1–40) or three (41–80) standardized and previously dried and weighed wooden sticks (115 mm × 10 mm × 2 mm) were buried horizontally at a depth of approximately 10 cm in MPs 1, 2, and 4 (Fig. 1). After a mean exposure time of 43 ± 3 d (range 9–100), the sticks were retrieved, washed, dried at 70°C for 48 h, and then weighed.

Microbial biomass.—At MPs 1, 2, and 4, mixed soil samples from five 10 cm deep soil cores of 1.6 cm diameter (approximately 30 g) were taken (Fig. 1). Soil microbial biomass carbon (MBC) was determined according to Vance et al. (1987) with the extraction of C from fumigated and unfumigated soils by K_2SO_4. An extraction efficiency coefficient of 0.38 was used to convert the difference in C between fumigated and unfumigated soil.

Statistical analyses

All statistical analyses were conducted with R (R Core Team 2016). We first investigated whether ecosystem function proxies differed among the five grassland types. Linear mixed-effects models were fit using the *nlme* package (Pinheiro et al. 2015, Bates et al. 2014) with random effects for field campaign and site. This is equivalent to a classical ANOVA with the additional advantage of accounting for the dependence of multiple measurements within plot and field campaign. For the ecosystem functions decomposition, invertebrate predation, and seed removal, which were measured multiple times per subplot, an additional random effect for within-plot sampling location was included. In the models for time-dependent ecosystem function assessments weight loss through decomposition, invertebrate predation, and abundance and biomass of pollinators, sampling duration was added as a covariate fitted before grassland type. If necessary, response variables were log- or square-root-transformed to improve variance homogeneity and normality of errors (indicated in Table 2). In the case of decomposition analyses, exposure

Table 2. Linear mixed models on the effect of five grassland types on ecosystem function proxies.

Ecosystem function	Proxy	Sampling duration	Grassland type
Aboveground primary productivity	Standing plant biomass†	–	$F_{4,74} = 17.36^{***}$
Aboveground secondary productivity	Number of invertebrates†	–	$F_{4,74} = 10.30^{***}$
	Fresh weight of invertebrates‡	–	$F_{4,74} = 7.25^{***}$
Belowground primary productivity	Root biomass‡	–	$F_{4,74} = 25.33^{***}$
Belowground secondary productivity	No. invertebrates†	–	$F_{3,75} = 2.74^{*}$
	Fresh weight of invertebrates†	–	$F_{4,74} = 2.67^{*}$
Pollination	No. pollinators†	$F_{1,159} = 21.09^{***}$	$F_{4,74} = 0.77$
	Fresh weight of pollinators†	$F_{1,159} = 2.18$	$F_{4,74} = 0.47$
Herbivory	Percentage of damaged leaves‡	–	$F_{4,74} = 16.79^{***}$
Invertebrate predation	Presence/absence of predation marks§	$X^2 = 0.07$†	$X^2 = 1.97$
Seed dispersal	Seed removal probability†	–	$F_{4,74} = 4.24^{**}$
Microbial biomass	Microbial biomass carbon†	–	$F_{4,73} = 3.53^{*}$
Decomposition	Weight loss through decomposition†	$F_{4,65} = 128.6$†***	$F_{4,65} = 4.88^{**}$

Notes: Field campaign, site, and multiplot/process sampling point (if applicable) were included as random effects. If measurement was time dependent, sampling duration was included as covariate (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

  † Log-transformed variable.
  ‡ Square-root-transformed variable.
  § Generalized linear model for logistic regression with X^2 and P values for likelihood-ratio tests after dropping the variable.

duration was log-transformed before the fitting of the model due to a decrease in daily weight loss rates over time. After fitting the linear mixed-effects models, pairwise differences among grassland types were tested using Tukey's post hoc test through the *glht* function from the *multcomp* package (Hothorn et al. 2008).

The model for invertebrate predation included a binary dependent variable for the presence of predation marks that was tested with a generalized linear mixed model for logistic regression using the function *lmer* in the *lme4* package (Bates et al. 2014). Random-effects structure was as for the other functions. Principal component analysis from the *vegan* package (Oksanen et al. 2015) was used to analyze the variation in ecosystem function levels within and among the five grassland types, using the per-site averages for each ecosystem function proxy as dimensions. Differences in individual ecosystem function levels between grassland types were further visualized in a schematic overview of a simplified ecosystem. Proportionate ecosystem function levels per grassland type were calculated for each ecosystem function by subtracting the lowest mean from all mean ecosystem function proxy levels and then dividing by the highest of those values. Line width in the schematic was proportional to this ratio, with three width categories corresponding to 0–33%, 34–66%, and 67–100% of the highest ecosystem function proxy level, respectively.

In a second part of the analysis, we investigated the effects of specific management components on ecosystem functions, including both historical land use and measures of current land-use practices. Linear mixed-effects models were fitted with the ecosystem function proxy as the dependent variable and historical land use and current management measures (grazing intensity, overseeding and fertilization, burning, and mowing) as fixed effects (in the given order). Fixed effects were preceded by sampling duration in the cases of time-dependent variables. A negative association between burning and overseeding (X_1^2 = 7.26, P = 0.007) and a positive association between overseeding and grazing intensity (t_{78} = 5.37, P = 0.007) were observed, and therefore, three different variable orders were tested for these variables. Random effects remained identical to those in the first set of mixed-effects models. Non-significant

terms were removed step-wise in order of least significance, resulting in minimum adequate models. Pairwise differences among historical land uses were established using Tukey's post hoc test as explained above.

RESULTS

Using rapid ecosystem function assessment

Rapid ecosystem function assessment methods allowed for sampling of ten different ecosystem function proxies with replication within site to be carried out by two people during one day per site (9 h). These short sampling times enabled a large-scale monitoring of ecosystem functions for a total of 80 grasslands of different types. Data collection on herbivory and seed removal was finalized directly in the field. Processing of samples for the standing plant and root biomass, predation dummies, and decomposition samples in the laboratory was quick (<1 h per site and ecosystem function). However, processing was more time-demanding for the pollinator and invertebrate samples, as well as the analysis of soil samples (up to several person-days for the cleaning and processing of the invertebrate samples). For time-dependent ecosystem function proxies, differing sampling durations were successfully corrected for in models. All REFA methods yielded usable proxies for the ecosystem functions of interest.

Effects of grassland type on ecosystem function proxies

Levels of most proxies for ecosystem functions differed significantly between several grassland types (Table 2, Fig. 2, random-effects estimates in Appendix S1: Table S3). Only the fresh weight and abundance of pollinators and the proportion of attacked dummy caterpillars did not show significant differences between grassland types. Aboveground standing plant biomass was significantly higher in low-intensity permanent grasslands (566 ± 73 g/m^2; mean ± SE) than in any other grassland type (Table 2, Fig. 2), reaching values three times higher than those in secondary grasslands after agriculture (180 ± 19 g/m^2). Root biomass was significantly higher in all permanent grasslands compared to both secondary grasslands (Fig. 2), without significant differences among the land-use types in each of the
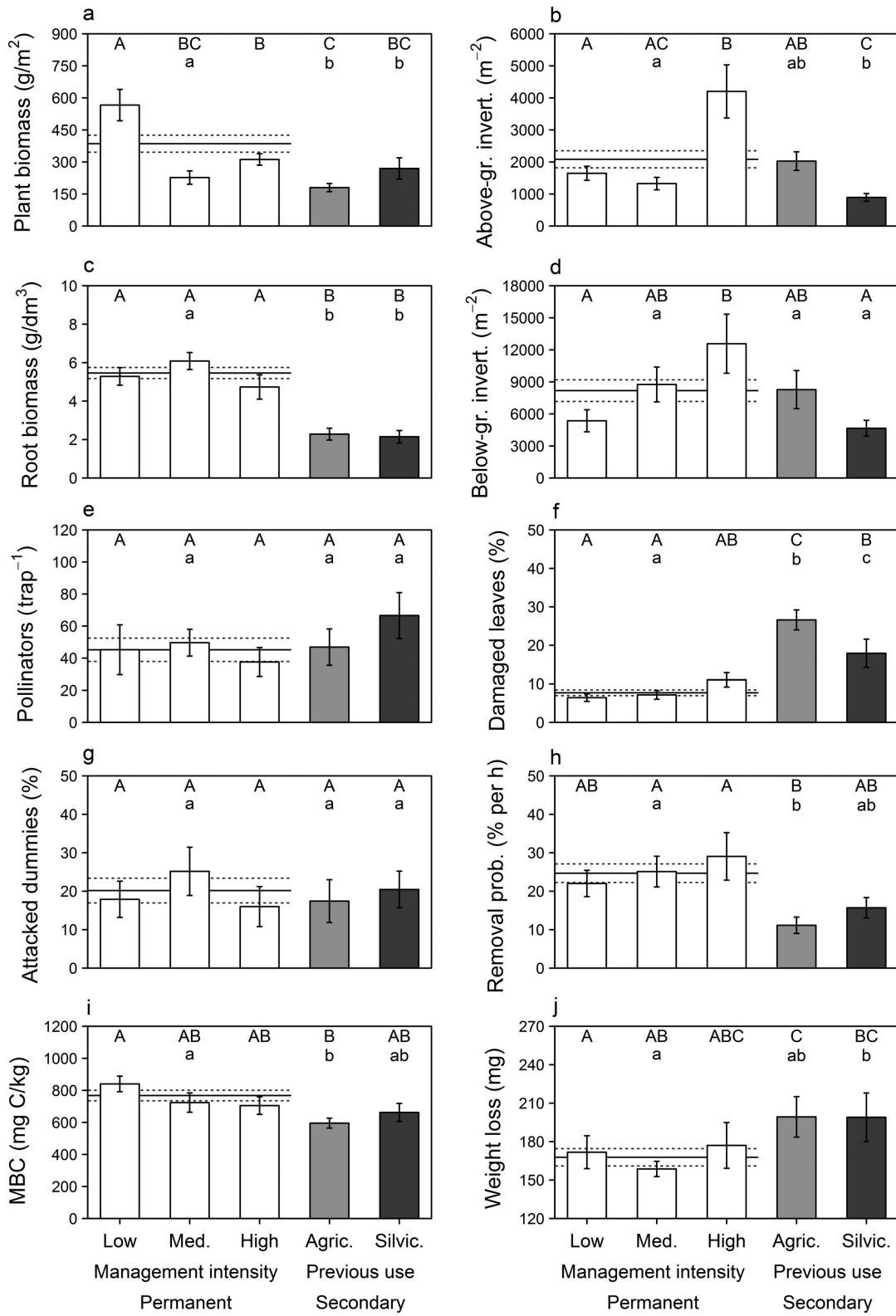
Fig. 2. Differences in ecosystem function proxies between grassland types. Ecosystem function proxies include

(Fig. 2. *Continued*)
the following: standing plant biomass (a), aboveground invertebrate abundance (b), root biomass (c), belowground invertebrate abundance (d), number of pollinators (e), share of leaves damaged by herbivory (f), share of attacked caterpillar dummies (g), seed removal probability (h), microbial biomass carbon (i), and weight loss of wooden sticks (j). Capital letters indicate differences between grassland types and lowercase letters differences between permanent and secondary grasslands, while the line indicates mean level of each ecosystem function across permanent grasslands.

two groups (Table 2). Medium-intensity permanent grasslands showed highest (6.1 ± 0.4 g/dm^3) and secondary grasslands after silviculture lowest values (2.1 ± 0.3 g/dm^3).

Abundance (4201 ± 828 individuals/m^2) of aboveground invertebrates was more than twice as high in high-intensity permanent grasslands compared to all other grassland types that had <2027 ± 290 individuals/m^2 (Fig. 2, Table 2). Abundance and fresh weight of invertebrates were positively correlated ($r = 0.58$, $n = 238$, $P < 0.001$), and the differences between grassland types showed the same but slightly less pronounced pattern when analyzing fresh weight instead of abundance (Appendix S1: Fig. S2). Similarly, the abundance of belowground invertebrates was highest in high-intensity permanent grasslands (12,572 ± 2765 individuals/m^2), significantly differing from the lowest abundances found in low-intensity permanent grasslands (5357 ± 1032 individuals/m^2) and secondary grasslands after silviculture (4655 ± 743 individuals/m^2; Fig. 2), exceeding these by a factor of 2.3 and 2.7, respectively. Fresh weight of belowground invertebrates correlated highly with abundance ($r = 0.59$, $n = 237$, $P < 0.001$) and was also highest in high-intensity permanent grasslands (Appendix S1: Fig. S2).

The percentage of leaves damaged by herbivores was significantly higher in secondary grasslands after agriculture (26.6 ± 2.6%) than in any other grassland type (Fig. 2, Table 2). Herbivory levels were intermediate on secondary grasslands after silviculture and on high-intensity permanent grasslands, and lowest on permanent grasslands with low and medium management intensity, that reached only around a quarter of the value found in secondary grasslands after agriculture (6.4 ± 1.0% and 7.1 ± 1.9%, respectively). Seed removal in high- and medium-intensity permanent grasslands differed significantly from secondary grasslands after agriculture (Fig. 2). The

average probability for a seed to be removed during 1 h in high-intensity permanent grasslands was 29.1 ± 6.2% and 25.1 ± 4.0% in medium-intensity grasslands, but in secondary grasslands after agriculture the probability was less than half (11.1 ± 2.1%). Seed removal probabilities in low-intensity permanent grasslands and on secondary grasslands after silviculture were intermediate. Out of 507 wooden sticks exposed to analyze weight loss through decomposition, 376 (74.2%) from 72 sites could be recovered. Of these, 32 were damaged during excavation and excluded from analysis. Total weight loss was significantly higher by >15% on secondary grasslands on former agricultural sites (199.3 ± 15.8 mg) compared to low- (171.7 ± 12.8 mg) and medium-intensity permanent grasslands (158.7 ± 5.9 mg; Fig. 2, Table 2), and on secondary grasslands after silviculture (199.0 ± 18.9 mg) compared to low-intensity permanent grasslands. Microbial biomass carbon was highest in low-intensity permanent grasslands (840.2 ± 48.6 mg C/kg) and lowest at only 71% of the former in secondary grasslands after agricultural use (595.5 ± 30.9 mg C/kg; Fig. 2, Table 2).

In contrast to the previously described ecosystem function proxies, the proxies for pollination and predation did not show significant differences between grassland types. The abundance and fresh weight of pollinators showed high variability in all grasslands, without significant differences between types. Numbers of individuals ranged from low single digits to >100 pollinators per PSP within each grassland type. Pollinator abundance increased with sampling duration, while the fresh weight of pollinators did not (Table 2). Of a total of 1560 exposed caterpillar dummies, 99.7% were recovered. On average, 19.6% of dummies per site showed signs of predation attempts. Most dummies were exclusively attacked by insects (95.7%), with only five attacks (2.1%) by each mammals or birds.

Hence, predator identity was not considered further in the analysis. Mean percentage of attacked dummies per site ranged from 16.5 ± 5.2% in high-intensity permanent grasslands to 25.9 ± 6.3% in medium-intensity permanent grasslands (Fig. 2), but neither grassland type nor duration of exposure showed any significant effect on the presence of predation marks on dummies (Table 2).

In summary, permanent grasslands of low intensity were characterized by a large plant biomass ecosystem component (above and below ground) that shrank under increased management intensity while the size of the consumer ecosystem component increased (Fig. 3). Secondary grasslands showed the smallest plant biomass ecosystem components and fastest decomposition (Fig. 3).

To characterize the combinations of ecosystem functions supported in the different grassland types, a principal component analysis was calculated using proxies for each of the investigated ecosystem functions as dimensions (Fig. 4). Permanent and secondary grasslands formed two distinct groups along the first principal component axis. Permanent grasslands were characterized by high plant biomass above and below ground, high MBC, and high seed removal potential, as well as low herbivory damage and low decomposition (i.e., weight loss), compared to secondary grasslands, confirming the significant differences between permanent and secondary grasslands found for these ecosystem functions. Within permanent and secondary grasslands, the grassland types did not separate out, and there was high variability along the second principal component axis. This axis was related to the proxies for predation, pollination, and abundance of aboveground invertebrates. Belowground invertebrate abundance was related to neither of the two-first principal component axes. These latter function proxies either did not show any significant differences between grassland types or showed largest differences among types within the groups of permanent or secondary grasslands.

Effects of historical land use and current management on ecosystem function proxies

Historical land use, that is, agriculture, afforestation, or management as grassland, had significant effects on almost all ecosystem function proxies for which the grassland types had significant effects (Table 3, random-effects estimates in Appendix S1:

| Low intensity | Medium intensity | High intensity | After agriculture | After silviculture |

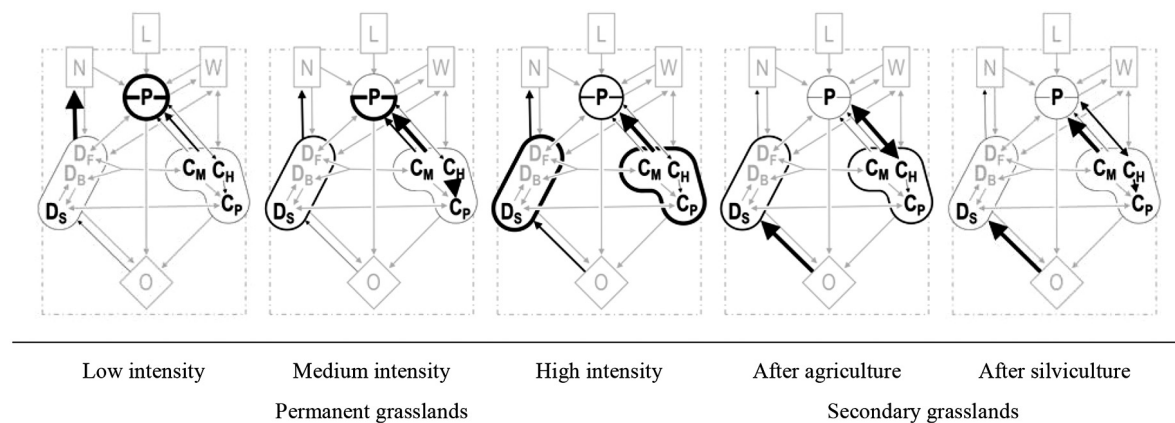| Permanent grasslands | | | Secondary grasslands | |

Fig. 3. Schematic representation of the different grassland types regarding the ecosystem functions supported. Line width is scaled proportionally to the highest measured function level in any type (wide, high; medium, medium; narrow, low). Shown are elements of the inorganic (rectangles), living (round shapes), and dead organic (diamond) ecosystem components, with the functions that link ecosystem components as arrows. Letters indicate pools nested within ecosystem components. These include primary producers (P); dead organic matter (O); decomposers (D), separated into fungi (D_F), bacteria (D_B), and saprophagous fauna (D_S); consumers (C), separated into microbial feeders (C_M), herbivores (C_H), and predators (C_P); nutrients N, P, K, and others (N); light (L); and water (W). Measured ecosystem functions are emphasized. Highlighted arrows indicate (clockwise from top left) mineralization (microbial biomass carbon as a proxy), pollination, seed removal, herbivory, predation, and decomposition.
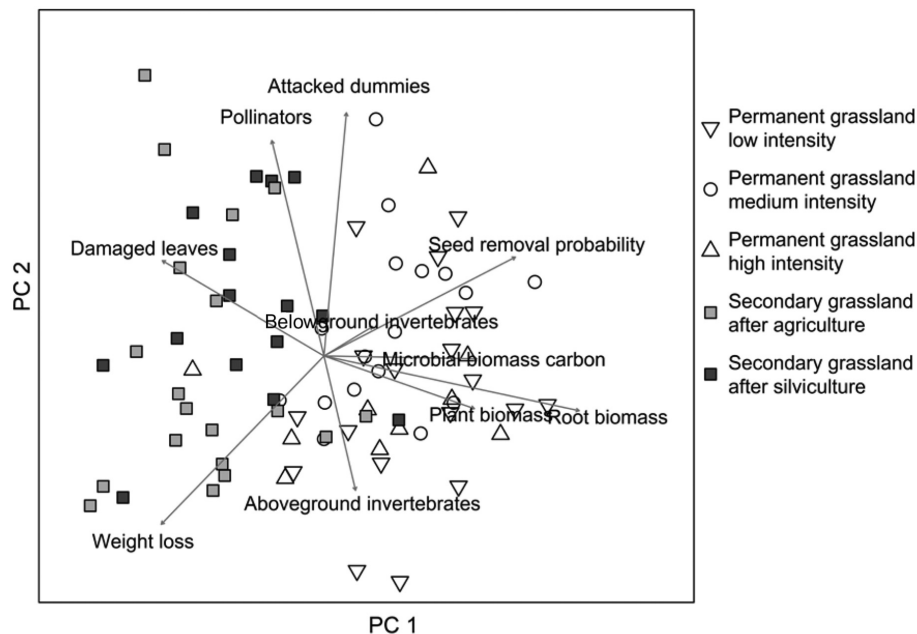
Fig. 4. Principal component analysis of the complete set of ecosystem function proxies of the 80 sites divided into five grassland types (triangle point down, permanent grassland with low-intensity management; circle, permanent grassland with medium-intensity management; triangle point up, permanent grassland with high-intensity management; filled square light gray, secondary grassland after agricultural use; filled square dark gray, secondary grassland after silvicultural use).

Table S4). Standing plant and root biomass were >40% or >139% higher in permanent grasslands (Fig. 2) compared to secondary grasslands, respectively. Aboveground abundance of invertebrates was also highest in permanent grasslands but differed significantly only from former pine plantations where values reached only 43% of those in permanent grasslands. Herbivory rates differed significantly between all three historical land-use types, being lowest in permanent grasslands at 7.69 ± 0.74% and about three times as high in former arable land at 26.6 ± 2.6%. Both seed removal probability and MBC were highest in permanent grasslands and lowest in former arable land, with intermediate values in former pine plantations. A single seed was more than twice as likely to be removed within 1 h in permanent grasslands compared to former arable land, while differences in MBC were less marked at 768 ± 33 mg C/kg in permanent grassland compared to 596 ± 31 and 662 ± 56 mg C/kg in former agricultural and silvicultural sites, respectively. Weight loss through decomposition, however, was more than 15% lower in permanent grasslands compared to

secondary grasslands but differed significantly only from former pine plantations.

All variables representing current management had significant effects on some function proxies (Table 3). Aboveground standing plant biomass decreased by 13.3% when grazing intensity as measured by heads of cattle months per hectare and year was doubled, while aboveground invertebrate abundance and fresh weight and belowground invertebrate abundance increased by 5.7%, 2.8%, and 20.1%, respectively (Fig. 5). Aboveground plant biomass and invertebrate abundance (Fig. 5) decreased by 38.1% and 40.9%, respectively—and root biomass more than doubled—on regularly burned sites. Like grazing intensity, overseeding and fertilizing positively impacted abundance and fresh weight of aboveground invertebrates, with nearly double the number of individuals in overseeded and fertilized grasslands. Overseeding was additionally associated with reduced root biomass and a 44.3% increase in the fresh weight of belowground invertebrates, and slightly but significantly higher weight loss through decomposition

Table 3. Linear mixed models on the effects of historical land use (natural grassland, agricultural use, pine plantation) and current management practices (grazing intensity, overseeding and fertilizing, burning, mowing) on ecosystem function proxies.

Ecosystem function proxy	Sampling duration	Historical land use	Grazing intensity†	Overseeding and fertilizing	Burning	Mowing
Standing plant biomass†	–	$F_{2,74} = 17.65^{***}$	$F_{1,74} = 14.48^{***}$	$F_{1,72} = 2.03^2$	$F_{1,74} = 23.69^{***}$	$F_{1,72} = 1.28^1$
No. invertebrates† (above ground)	–	$F_{2,74} = 11.36^{***}$	$F_{1,74} = 4.22^*$	$F_{1,74} = 6.30^*$	$F_{1,73} = 3.21^2$ ($F_{1,74} = 6.08^*$)	$F_{1,72} = 2.69^1$
Fresh weight of invertebrates‡ (above ground)	–	$F_{2,74} = 6.42^{**}$	$F_{1,74} = 7.58^{**}$	$F_{1,74} = 6.40^*$	$F_{1,73} = 1.66^2$ ($F_{1,74} = 3.98^*$)	$F_{1,72} = 0.80^1$
Root biomass‡	–	$F_{2,75} = 49.01^{***}$	$F_{1,72} = 1.49^1$	$F_{1,75} = 5.92^*$	$F_{1,73} = 2.93^2$ ($F_{1,74} = 5.56^*$)	$F_{1,74} = 3.01^3$ ($F_{1,74} = 4.00^*$)
No. invertebrates† (below ground)	–	$F_{2,75} = 1.22^4$	$F_{1,77} = 5.70^*$	$F_{1,73} = 0.32^2$	$F_{1,74} = 0.98^3$	$F_{1,72} = 0.31^1$
Fresh weight of invertebrates† (below ground)	–	$F_{2,74} = 1.09^3$	$F_{1,76} = 3.14^4$	$F_{1,77} = 7.79^{**}$	$F_{1,73} = 0.70^2$	$F_{1,72} = 0.61^1$
No. pollinators†	$F_{1,159} = 21.32^{***}$	$F_{2,75} = 1.09^4$	$F_{1,77} = 0.33^5$	$F_{1,74} = 0.31^3$	$F_{1,73} = 0.24^2$	$F_{1,72} = 0.04^1$
Fresh weight of pollinators†	$F_{1,159} = 2.23^6$	$F_{2,76} = 0.91^5$	$F_{1,73} = 0.02^2$	$F_{1,74} = 0.02^3$	$F_{1,72} = 0.00^1$	$F_{1,75} = 0.39^4$
Percentage of damaged leaves‡	–	$F_{2,76} = 29.48^{***}$	$F_{1,72} = 0.00^1$	$F_{1,75} = 2.95^4$	$F_{1,73} = 0.27^2$	$F_{1,74} = 0.60^3$
Presence/absence of predation marks§	$X^2 = 0.02†^3$	$X^2 = 0.64^4$	$X^2 = 0.11^2$	$X^2 = 0.56^1$	$X^2 = 2.30^5$	$X^2 = 10.61^{**}$
Seed removal probability†	–	$F_{2,75} = 8.17^{***}$	$F_{1,74} = 2.66^3$	$F_{1,73} = 0.15^2$	$F_{1,72} = 0.04^1$	$F_{1,75} = 4.17^*$
Microbial biomass carbon†	–	$F_{2,75} = 4.73^*$	$F_{1,72} = 0.43^2$	$F_{1,71} = 0.03^1$	$F_{1,74} = 1.52^4$	$F_{1,73} = 0.62^3$
Weight loss†	$F_{1,66} = 128.4†^{***}$	$F_{2,66} = 6.80^{**}$	$F_{1,65} = 1.76^3$	$F_{1,66} = 5.71^*$	$F_{1,63} = 0.04^1$	$F_{1,64} = 1.56^2$

Notes: Superscript numbers indicate order of deletion. Field campaign, site, and multiplot/process sampling point (if applicable) were included as random effects. If the measurement was time dependent, sampling duration was included as a covariate. Grazing intensity, burning, and overseeding and fertilizing were correlated; therefore, F values in parentheses indicate significance after alterations of the order of these variables ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).
  † Log-transformed variable.
  ‡ Square-root-transformed variable.
  § Generalized linear model for logistic regression with X^2 and P values for likelihood-ratio tests after dropping the variable.

(Fig. 5). Mowing significantly increased the probability of a caterpillar to bear predation marks, with double the share of marked caterpillars on mown sites compared to unmown sites. Additionally, the removal probability of sunflower seeds was 43.9% higher in mown sites (Fig. 5). Changing the variable order for overseeding and fertilizing, grazing intensity, and burning in the models, affected which management practices showed significant effects on the ecosystem function proxy number and fresh weight of aboveground invertebrates, root biomass, and number of belowground invertebrates (Table 3; Appendix S1: Tables S1 and S2). This indicates that some ecosystem functions are affected by several correlated management practices without being able to attribute the effect to a single practice.

Discussion

The highly standardized REFA procedures yielded usable proxies of sufficient resolution to detect land-use effects for many of the surveyed ecosystem functions. It is important to emphasize that REFA methods measure standardized proxies for the ecosystem functions of interest rather than the functions themselves. Also, some caveats became apparent during the measurements. The approximation of primary productivity by standing biomass was complicated in sites where low management intensity causes the accumulation of large amounts of dead biomass. At these sites, standing biomass, which includes dead biomass, produced an inflated estimate of primary productivity. Likewise, in sites with high grazing intensity, standing biomass
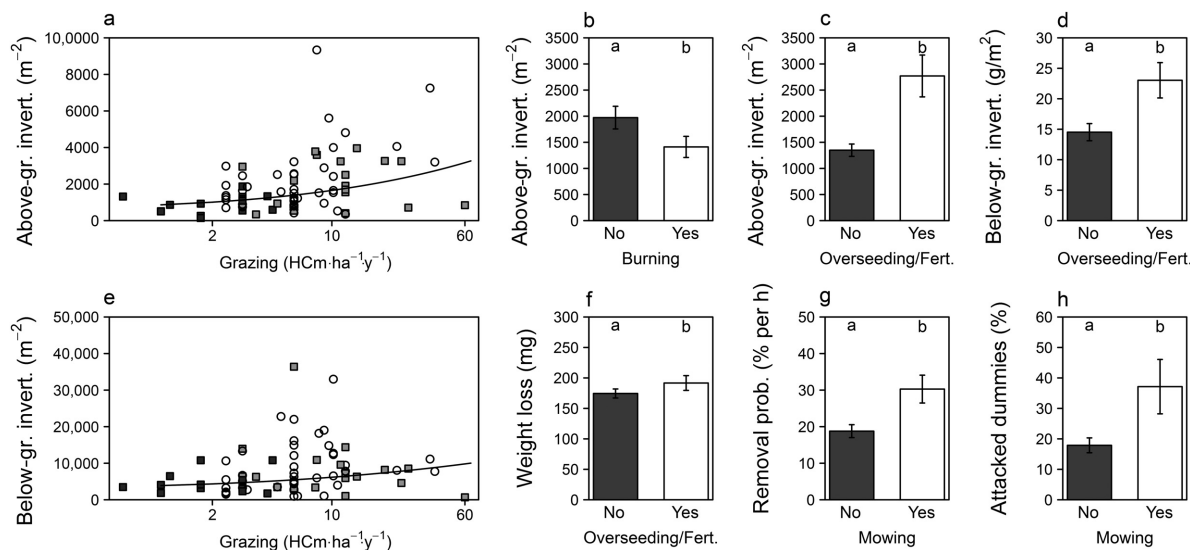
Fig. 5. Impact of the continuous variable "grazing intensity" (heads of cattle months per hectare and year, note: x-axis is log-transformed; a, e) and the binary variables "burning" (b), "overseeding and fertilizing" (c, d, f), and "mowing" (g, h) on selected ecosystem function proxies (only significant effects shown). Lowercase letters indicate significant differences (circle, permanent grassland; filled square light gray, secondary grassland after agricultural use; empty square dark gray, secondary grassland after silvicultural use).

underestimates productivity. Consequently, the methods to approximate primary productivity should be adapted when working in grazed (e.g., fencing) or abandoned sites (e.g., measuring green biomass only). Processing of the invertebrate samples in the laboratory was far more time-consuming than the other proxy measurements. Future studies using REFA methods should take this into account when choosing the ecosystem function proxies of interest. Lastly, some decomposition samples were lost because of the destruction of markings by cattle and resulting failure to locate their positions. More robust marking could reduce the risk of losing samples in future studies.

For most ecosystem functions, grassland type, historical land use, and one or more current management practices showed significant effects. Individual ecosystem functions responded differently to the explanatory variables, consistent with previous reports of contrasting effects of land-use intensity on functions attributed to different types of ecosystem services (Allan et al. 2015). The five main grassland types in the study region showed noticeable differences between

supported ecosystem functions. These differences were especially large between permanent and secondary grasslands. Historical land use, that is, the distinction between permanent grasslands that have never been converted and re-established grasslands after agricultural or silvicultural use, had significant effects on seven of the ten ecosystem functions tested. Root biomass, MBC, and seed dispersal were higher in permanent grasslands. Increased herbivory was found in secondary grasslands and higher decomposition rates in former pine plantations compared to permanent grasslands.

These results underline the lasting effects of former agricultural and silvicultural use even after reconversion to grassland, in line with the findings by Koch et al. (2016), who found that floristic composition differed most strongly between permanent and secondary grasslands. Land-use history is known to be a major factor in determining present-day conditions, and its general influence on individual ecosystem functions including productivity, soil structure and chemistry, and microbial activity is well documented (Foster et al. 2003). Soil properties such as

nutrient availability and plant species composition of restored grasslands have been found to recover only slowly from periods of agricultural or silvicultural use (Baer et al. 2002, Walker et al. 2004, McLauchlan et al. 2006). Baer et al. (2002) showed that grasslands were more productive while having reduced soil organic carbon and root biomass after agricultural use. These findings are in line with high cattle carrying capacities and our results of reduced root biomass in these grasslands. It takes decades for soil organic carbon content in ex-arable land to return to levels found in permanent grasslands (McLauchlan et al. 2006). A low availability of organic material affects the ecological functions that soils provide either directly or indirectly through effects on soil invertebrates (Lavelle et al. 2006) or the soil microbial community (van Veen et al. 1989). Overall low levels of herbivory in permanent grasslands can be explained by grass dominance, as indicated by findings by Koch et al. (2016) of lower graminoid cover in secondary grasslands. Grasses generally experience less damage from herbivores than forbs (Loranger et al. 2013, Gossner et al. 2014). Finally, seed removal was highest in permanent grasslands, likely explained by higher ant activity compared to secondary grasslands. Ants are important seed predators in grasslands (Beattie 1989).

Ecosystem functions differed not only between permanent and secondary grasslands but also among grasslands of the same type but under different management. This indicates that current management practices are also relevant to ecosystem functions, as confirmed in the subsequent models. Management practices such as fertilization, burning, and grazing impact ecosystem functions either directly through changes in abiotic conditions, or indirectly via input of dung or by triggering shifts in vegetation composition (Allan et al. 2015). Aboveground primary productivity, measured by peak standing biomass, was highest in low-intensity permanent grasslands but did not differ between the other grassland types. This finding, at first sight, contrasts with numerous previous findings that have shown significant positive effects of grazing and burning on grassland primary productivity (Semmartin and Oesterheld 1996, 2001, Oesterheld et al. 1999), especially compared to abandoned grasslands where accumulated biomass

limits light availability for emerging plants (Knapp 1984). However, this difference can be explained by our use of standing plant biomass including senescent biomass as a proxy for primary productivity. In permanent grasslands of low management intensity, large amounts of dead grass biomass accumulate due to a lack of grazing and burning. Above- and belowground invertebrates peaked in high-intensity permanent grasslands, surpassing the abundances in low-intensity permanent grasslands despite significantly larger amounts of plant biomass in the latter. This may be explained by differences in plant species composition, more specifically a larger proportion of high-quality forage species and plants with higher nitrogen content in overseeded grasslands being able to sustain larger consumer communities. Generally, positive effects of overseeding and fertilization, grazing intensity, and burning on invertebrate abundance and fresh weight are most likely a consequence of increased resource quality and availability due to stimulated new growth and increased nitrogen availability. Increased richness and abundance of grassland arthropods due to fertilization have been shown before (Siemann 1998). Slightly faster decomposition on fertilized sites may also be a consequence of nitrogen limitation in early stages of decomposition (Hobbie 2005), although the influence of nitrogen on decomposition can be positive, neutral, or negative depending on substrate quality and the amount of fertilizer (Knorr et al. 2005). Higher root biomass on burned sites is consistent with previous findings from the same study area, that emphasized the importance of bud-bearing organs for plants on sites with frequent fire occurrences (Fidelis et al. 2009, 2014). The increase in seed removal and invertebrate predation caused by mowing might be a consequence of simplified vegetation structure or arthropod assemblages that differ from those in burned areas (Swengel 2001).

Besides historical land use and current management, also larger-scale landscape effects, as well as small-scale spatial heterogeneity or vegetation community composition, can affect levels of ecosystem functioning. Failing to include these variables in our study is potentially the reason for the large amounts of unexplained variance observed in predation, pollination, and seed

dispersal. In pollinators, for example, floral richness (Potts et al. 2009) and landscape context at spatial scales of up to several kilometers (Steffan-Dewenter et al. 2002) can affect foraging patterns. In predators, small-scale habitat heterogeneity is known to influence invertebrate assemblages (Reid and Hochuli 2007) and predation pressure on invertebrates (Langellotto and Denno 2004), potentially outweighing differences between grassland types.

Conversions between land-use types in our study region, in addition to changes in biotic and abiotic composition, caused marked changes in ecosystem functions which persisted for many years after reconversion to grasslands. Compared to the conversion to agriculture or silviculture, effects of current management practices on ecosystem functioning were less severe. However, processes linked to species interactions such as predation and seed dispersal, but also secondary productivity, were most sensitive to current management practices. While all management practices (burning, grazing, fertilizing combined with overseeding, and mowing) showed significant impacts on some of the investigated ecosystem functions, effects were less pronounced than previously documented changes in plant community composition due to repeated fertilization and the introduction of exotic species (Koch et al. 2016). This difference between effects on vegetation composition and ecosystem functions is most apparent when comparing low-intensity with medium-intensity permanent grasslands. While a decrease in plant biodiversity and a shift in species composition have been demonstrated for the low-intensity grasslands (Koch et al. 2016), only small and non-significant differences in ecosystem functioning were found between these two types. This might indicate a certain degree of robustness of the investigated ecosystem functions to effects of management practices and resulting plant species loss, either because of the conservation of relevant functional traits or because of strong dependencies on abiotic environmental conditions that are largely maintained under less intensive land use (Hooper et al. 2005). Consequently, studies investigating land-use effects, or evaluating the success of restoration or conservation actions, should not rely on monitoring only biotic composition or ecosystem functions but should combine the monitoring of both

to avoid missing important effects (Kollmann et al. 2016). Only when understanding the effects of land-use changes on biodiversity and ecosystem functioning, can the provisioning of ecosystem services by natural and semi-natural habitats be quantified and considered in relevant decision processes (Millennium Ecosystem Assessment 2005). Importantly, these considerations need to take into account the long-lasting legacy effects of land-use changes on the provisioning of ecosystem functions and services documented here. To preserve the biotic composition and the functioning of native grasslands in southern Brazil, a significant portion of grasslands needs to be kept under traditional management. Conservation of these grasslands would also ensure the continued provisioning of numerous important ecosystem services (Sala and Paruelo 1997).

CONCLUSION

Our study, for the first time, provides a quantification of the impacts of historical land-use change and intensification of current management on a comprehensive set of ecosystem functions in South Brazilian grasslands. Historical land-use change involving conversion to agricultural or silvicultural use causes lasting changes in ecosystem functions even after reconversion to secondary grasslands. Return to pre-conversion functioning and species composition appears to be, at best, slow and may even be impeded by alternate stable ecosystem states. Land-use intensification can also boost certain ecosystem functions on permanent grasslands, mostly those linked to productivity, while the abandonment of land-use practices proved to be less of a threat to the surveyed ecosystem functions than it is to plant species composition and diversity. Traditional grassland management including burning and extensive grazing sustains ecosystem functions and species diversity while contributing to cattle production, offering a more sustainable alternative to high-intensity land-use forms that harm ecosystem functioning and lastingly alter abiotic and biotic conditions. The vast grasslands of southeastern South America are an asset worth conserving, and even temporary conversions to other land-use types cause a long-term impairment of both biodiversity and ecosystem

functioning, most likely affecting the goods and services these grasslands provide.

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