

Relative contribution of soil, management and traits to co-variations of multiple ecosystem properties in grasslands

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Abstract Ecological intensification promotes the better use of ecosystem functioning for agricultural production and as a provider of additional regulation and cultural services. We investigated the mechanisms underpinning potential ecological intensification of livestock production in the Vercors mountains (France). We quantified the variations in seven ecosystem properties associated with key ecosystem services: above-ground biomass production at first harvest, fodder digestibility, plant species richness, soil organic matter content, soil carbon content, total microbial biomass and soil bacteria:fungi ratio across 39 grassland plots representing varying management types and intensity. Our analyses confirmed joint effects of management, traits and soil abiotic parameters on variations in ecosystem properties, with the combination of management and traits being most influential. The variations explained by traits were consistent with the leaf economics spectrum model and its implications for ecosystem functioning. The observed independence between ecosystem properties relevant to production (forage biomass, digestibility and nutrient turnover) on the one hand and soil stocks (organic matter, carbon and microbial stocks) on the other hand suggests that

an intensification of fodder production might be compatible with the preservation of the soil capital. We highlight that appropriate choices regarding various practices, such as the first date of grazing or mowing being dependent on soil moisture, have important consequences on a number of ecosystem properties relevant for ecosystem services and may influence biodiversity patterns. Such avenues for ecological intensification should be considered as part of further landscape- and farm-scale analyses of the relationships between farm functioning and ecosystem services.

Keywords Biodiversity · Ecological intensification · Flowering phenology · Leaf economics spectrum · Mountain grassland

Introduction

The consequences of land use change on the biodiversity and functioning of the ecosystems have received growing attention since the end of the 20th century, coupled to strong warnings about a possible shift in ecosystems' states (Barnosky et al. 2012) and subsequent degradation of ecosystem services and human well-being (Cardinale et al. 2012; Hooper et al. 2012). Agriculture has been pointed out as one of many factors responsible for causing environmental damage that may result in long-term losses in ecosystem services, including many that support agriculture itself (Foley et al. 2005). Among other solutions, 'ecological intensification' of agriculture, defined as 'all the processes of transformation of productive ecosystems towards higher yields produced with reduced forcing of the agroecosystems' (Griffon 2009), would reconcile the conflicting challenges of increasing global food production to meet the needs of a projected human population of 9 billion by 2050

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[Food and Agricultural Organisation of the United Nations (FAO), Geneva, Switzerland] on the one hand and of reducing impacts on ecosystems on the other hand. Although the concept of ecological intensification has primarily been studied in the context of research on intensive cropping systems (Cassman 1999), more recently it has been also applied to other farming systems, including livestock farming (Griffon 2009). This new paradigm would address recent criticisms of livestock farming due to its strong negative environmental impacts (especially climate change through greenhouse gas emissions; Steinfeld et al. 2006), concurrent with recent and projected dramatic increases in demand for animal—especially meat—products worldwide (FAO).

While progress in plant and animal sciences and genetic engineering has been seen as one of the key pathways to ecological intensification (Doré et al. 2011), ‘ecological intensification’ also strongly emphasises the better use of ecosystem functioning as input services (sensu Zhang et al. 2007) for agricultural production and as a provider of additional regulation (e.g. climate regulation) and cultural (e.g. aesthetic value) services from agro-ecosystems to society at large (Le Roux et al. 2009; Bommarco et al. 2013).

Because ecosystem services are not independent of one another and are provided as ‘bundles’ (MEA 2005), formally defined as ‘a set of ecosystem services that repeatedly appear together across space or time’ (Raudsepp-Hearne et al. 2010), assessing the provision of multiple ecosystem services, including their positive or negative associations and their interactions with biodiversity, was highlighted by the Millennium Ecosystem Assessment as one of the major scientific challenges (Carpenter et al. 2009) and is a current research priority (e.g. Crossman et al. 2013; Nagendra et al. 2013; Bennett et al. 2015). The new challenge of explicitly analysing ecosystem service bundles (Bennett and Balvanera 2007; Seppelt et al. 2012; Carpenter et al. 2009) is expected to shift research practice from a first generation of assessments (considering only ≤ 5 ecosystem services simultaneously and seldom displaying interactions between ecosystem services; Seppelt et al. 2011) to new standards where most studies consider broad bundles of ecosystem functions and services (Burkhard et al. 2012). This challenge should in particular be endorsed by research on ecological intensification of agriculture, with an emphasis on the increase in multiple regulation, provisioning and even cultural services (Bommarco et al. 2013).

In the study reported here, we investigated the ecosystem properties underpinning the supply of ecosystem services by grasslands in a French pre-Alps region. Livestock production in this area is currently facing the conflicting objectives of biodiversity conservation (linked with the registered designation of origin of a local cheese) and milk production to supply the local dairy cooperative (Dobremez

et al. 2015). Our objective was to identify bundles of ecosystem properties and their influencing factors at the scale of individual grasslands. We analyzed the variations in seven ecosystem properties associated with the provision of fodder and underlying soil-based regulating services, including above-ground biomass production at first harvest (ABM), fodder digestibility, plant species richness (SR), soil organic matter content (SOM), soil carbon (C) content, total phospholipid fatty acid (PLFA) as a proxy for total microbial biomass (TMB) and soil bacteria:fungi ratio (BFR). ABM and hay digestibility are two ecosystem properties which are of direct interest to livestock farmers who put priority on one or the other, or both, with respect to individual grasslands within their farms; alternatively, plant SR is an indicator addressing biodiversity objectives. Soil C content was incorporated as an indicator of climate regulation, while SOM and microbial parameters were considered as indicators of the maintenance of soil fertility.

Based on a previous study at another mountain grassland site, which demonstrated the relevance of grassland management, soil properties and plant traits to variations in individual ecosystem services (Lavorel et al. 2011), we quantified the relative effects of these three groups of parameters and their interactions on the joint variations in the seven target ecosystem properties. We hypothesised that: (1) grassland management strongly determines the joint variations in the seven ecosystem properties and, thereby, of associated ecosystem services; (2) consistent with Lavorel et al. (2011), plant traits make an important contribution to the explanation of the multivariate variations in ecosystem properties; (3) below-ground parameters interact with above-ground parameters in determining grassland ecosystem properties (Grigulis et al. 2013). Our results are discussed in the context of their significance to ecological intensification of grassland production for livestock farming.

Materials and methods

Study site and field measurements

The study site (45°07'N, 5°31'E) is located in the French Pre-Alps, in North Vercors, on the plateau of Méaudre. It covers a total area of 78 km², and the elevation of the sampled plots ranged from 930 to 1311 m a.s.l. Based on evidence that land use legacies strongly influence current soil properties, vegetation communities and ecosystem functioning (Quétier et al. 2007a) and assuming that the stability of the current land use over the past decades, we sampled 39 plots distributed across different grassland types. Grasslands were classified according to a preexisting typology based on the visual frequency of different plant

Table 1 Classification of the 39 grasslands plots with their main management characteristics

Type of use	Grassland type ^a	Code ^b	Main use	Management regime ^c	Altitudinal range (m a.s.l.)	Number of plots	% Landscape
Hay meadows (M)	Grass- or legume-dominated intensive grasslands	M1	Mowing	Sowing <12 years 70 kg N ha ⁻¹ year ⁻¹ Cut ≥2 times/year	984–1048	11	16
	Grass-dominated permanent grasslands	M3	Mowing	Sowing >12 years 70 kg N ha ⁻¹ year ⁻¹ One cut, 1 grazing	993–1078	5	19
	Forb-dominated permanent grasslands	M4	Mowing	Sowing >12 years 70 kg N ha ⁻¹ year ⁻¹ One cut, 1 grazing	999–1157	5	18
	Grass-dominated extensive permanent grasslands	M5	Mowing	Sowing if any >25 years 30 kg N ha ⁻¹ year ⁻¹ One cut, 1 light grazing	987–1041	4	2
	Forb-dominated extensive permanent grasslands	M6	Mowing	Sowing if any >25 years 30 kg N ha ⁻¹ year ⁻¹ One cut, 1 light grazing	1085–1149	2	2
	Pastures (P)	Grass- and legume-dominated intensive pastures	P1	Dairy cows and/or heifer grazing	Intensive grazing from April–May 8 kg N ha ⁻¹ year ⁻¹	988–1195	10
Grass-dominated extensive pastures		P5	Dairy cows and/or heifer grazing	Extensive grazing from June 0 kg N ha ⁻¹ year ⁻¹	988–1024	2	8

^a Studied grasslands are classified according to (1) their main use (type of use) and (2) their grassland type based on the GIS Alpes du Nord typology (2002)

^b See “Study site and field measurements” section for explanation of the code for the six subtypes of mown (M1–M3) and pastured (P1–P3) grasslands

^c Management regime for each grassland type as described by: time since last sowing for mown grassland/grazing intensity for pastures, median annual fertiliser (N, nitrogen) input and use regime

types (GIS Alpes Du Nord 2002), with the aim to define mountain grassland ‘use value’—i.e. grassland quality with respect to the expectations of the farmer. This typology was originally derived by agronomers according to four suitability criteria that were used to qualitatively assess test parcels throughout the northern French Alps: (1) dynamics of above-ground biomass during plant growth and regrowth; (2) temporal dynamics of hay nutritional food value [hay nitrogen (N) content and digestibility]; (3) hay harvest suitability (drying needs, losses during haying); (4) vegetation community dynamics (Jeannin et al. 1991). In the field, grasslands are assigned to classes of the typology based on plot-scale visual estimates of two empirical indicators of management regimes: (1) the percentage of non-legume dicots, which is used as an indicator of management intensity as it increases with late mowing and increases with fertilisation; (2) the physiognomy of dominant grasses as integrators of soil nutrient and environmental water availability, where greater fertilisation favours bunch versus diffuse grasses. The typology is therefore an indicator of

management intensity relating to dominant practices on a given plot, although there may be some inter-annual variability in specific practices. In order to facilitate the initial sampling, and especially because some categories may be difficult to distinguish in the field, we simplified this original grassland typology. Using expert knowledge and field observations by agronomists, we distinguished mown and pastured grasslands and then divided each category into six subtypes (M1–M6 and P1–P6, respectively) ranked by decreasing intensity of use. Table 1 describes the average management regimes and corresponding indicators for each grassland type. Consistent with the frequency of different grassland types in the landscape, our sample only contained types M1, M3–M6, P1 and P5, and the representation across grassland types was uneven (Table 1). Sampling pressure was also increased for type M1 during the second season based on observed high heterogeneity between plots. Because plot-level values for fertiliser input and grazing and mowing regimes were not available for all plots as continuous explanatory variables, we then

used these grassland types as proxies for management. We also assessed the date of first mowing or grazing through weekly surveys. Field sampling was completed over two vegetation seasons, 2011 (27 plots) and 2012 (21 plots), the latter comprising nine repeated plots distributed across grassland types for calibration across the 2 study years. Soil and vegetation nutrition indices were measured during the vegetative state (May–early June) and other parameters just prior to first use, corresponding essentially to peak vegetation.

Soil and nutrient availability

Five 10-cm-deep soil cores were randomly sampled in each plot at the date of fully developed swards (100 % cover; end of April in 2011, end of May in 2012), then pooled together prior to analysis. Soil texture and soil total C and N content were measured following previously described protocols (Grigulis et al. 2013). Soil water-holding capacity (WHC) and water availability (WA = WHC – permanent wilting point) were calculated using texture and total C data (Ostý 1971).

Fresh, sieved (i.e. 5.6-mm mesh) soil samples were initially stored at $-20\text{ }^{\circ}\text{C}$ (for further PLFA analysis) or at $4\text{ }^{\circ}\text{C}$ and within 48 h processed for microbial community analysis and soil chemical analysis, respectively. Soil water content was determined on fresh soil dried at $70\text{ }^{\circ}\text{C}$ for 1 week. Total soil C and N content was determined in soil subsamples that had been air dried and finely ground using a FlashEA 1112 elemental analyzer (Fisher Scientific, Waltham, MA). Soil pH was measured using a 1:4 (soil:distilled water) solution. Soil density was obtained measuring the dry mass of a fixed volume soil core. Soil nutrients [ammonium ($\text{NH}_4\text{-N}$), nitrate ($\text{NO}_3\text{-N}$), total dissolved N and dissolved organic N] were measured from 0.5 M K_2SO_4 soil extracts (Jones and Willett 2006), then analyzed on a FS-IV colorimetric chain (OI-Analytical Corp., College Station, TX).

A principal component analysis (PCA) of soil parameters identified three main axes explaining 46, 19 and 12 % of the variation of the dataset, respectively. The first axis was significantly associated with water availability (WHC, wilting point), soil granularity (clay and sand content) and the physical parameters associated with clay content [cation exchange capacity (CEC), pH, soil N content] [Electronic Supplementary Material (ESM) Table S1]. The second axis was significantly associated with water availability and silt. The third axis was significantly associated with soil phosphorus (P) content and apparent density. We used these soil-PCA axes as integrated soil abiotic parameters. In addition to these soil measurements, N and P nutrition indices (NNI and PNI, respectively) were measured in each plot to quantify actual nutrient availability to plant growth

(Lemaire and Gastal 1997). Briefly, using standard protocols (Garnier et al. 2007), we sampled above-ground live biomass in four 0.25-m^2 quadrats at the vegetative stage. Live dicots and grasses were separated from legumes. NNI was calculated as the ratio between the actual N concentration of above-ground biomass and the critical N concentration (i.e. concentration allowing potential growth; Lemaire and Gastal 1997). PNI was calculated as proposed by Duru et al. (1997) and Jouany et al. (2004).

Vegetation data

Floristic inventories

All species were recorded within each plot and the relative abundance of each species calculated using the point-quadrat sampling method (Levy and Madden 1933). For a given plot, the local abundance of each species was determined as the number of hits among 160 sampling points evenly distributed within four $50 \times 50\text{-cm}^2$ and two $2 \times 2\text{-m}^2$ quadrats located randomly within the plot.

Plant vegetative traits

Those plant vegetative traits [vegetative height (VegH), inflorescence exposition (FE), leaf dry matter content (LDMC), leaf N, C and P concentrations (LNC, LCC and LPC)] assumed to be relevant to ecosystem service provision (Lavorel et al. 2011) were measured following standard protocols at maximum biomass (Cornelissen et al. 2003; Garnier et al. 2007). Briefly, during the 2012 season within each grassland type, for each species contributing to the cumulated 80 % of biomass, (1) the vegetative and reproductive heights (with inflorescence exposition as their difference) of 20 individuals were measured, and (2) the last mature leaf of 10 individuals was collected, prepared and measured for LDMC, LNC, LCC and LPC. Because grassland management is a primary source of intraspecific trait variability within a given site, we repeated trait measurements for each species within each grassland type, with the sampled individuals distributed across plots for that type (Garnier et al. 2007). For each trait within each plot, we calculated the community-weighted mean (CWM) value for that trait (Garnier et al. 2004), where the trait value for each species within the corresponding grassland type was weighted by its relative abundance in the plot using the FD package (Laliberté and Shipley 2011).

Botanical survey data were also used to calculate the abundance of early- and late-flowering grass species, the abundance of conservative grass species and the abundance of exploitative grass species, following the perennial grass species typology of Cruz et al. (2010). This method uses the values of six functional characteristics that discriminate

among the agricultural qualities of grass species, namely, dry matter content, specific leaf area, life span and toughness for leaves and flowering date and maximum height for the whole plant.

Flowering phenology

Date of flowering onset was surveyed for all abundant grass species in 2011 (27 plots) and 2012 (21 plots). The restriction of this trait to grass species is based on the convergence in the timing of flowering between grass and dicots within a given community (Ansquer et al. 2009) and on the overall greater relevance to farmers of grass flowering as a marker for a switch in the grassland towards a lower quality state (Duru et al. 2010). For each species, the phenological state (vegetative, ears in their sheath, developed ears and flowering ears) was determined once weekly as the dominant state of the population. Data were obtained from the 38021001 Autrans Météo France station, and date of flowering onset was transformed to growing degree-days adjusted to altitude for each plot by applying a 0.6 °C/100 m decline. Flowering dates expressed in degree-days were cross-calibrated across the 2 years of measurements using a linear regression for the nine calibration plots, with 2012, a climatically average year, used as the baseline.

Ecosystem properties

Seven variables were selected as ecosystem properties associated with ecosystem services: ABM, digestibility, soil C content, SOM, TMB and BFR (see "Introduction" for explanation of abbreviations), as well as plant SR, respectively associated with the following ecosystem services hay quantity and quality, C storage and retention of nutrients in soil (Lavorel et al. 2011; Grigulis et al. 2013).

ABM at first harvest was estimated using calibrated height measurements (Lavorel and Grigulis 2011). Briefly, a calibration equation ($R^2 = 0.721$, $p < 0.001$) was established in 2011 between the average sward height (i.e. the height of the grassland canopy excluding stems and flower heads) of four of 12 measured quadrats per plot and harvested, sorted (live vs. litter) and dried biomass. Given its robustness across years and sites (Lavorel and Grigulis 2011), we applied the same equation in our study for both 2011 and 2012. ABM was also calibrated by a linear regression across the 2 years using the nine repeated plots, with 2012, a climatically average year, used as the baseline. Digestibility of green biomass was measured at the maximum growing rate of vegetation, namely, at the end of June, on a 1-m² sample of vegetation within each plot cut on four 50 × 50-cm quadrats; the biomass was sorted, dried for 72 h at 60 °C, ground with a 0.5-mm mill and analyzed with near-infrared spectrometry to determine total

N content and digestibility (calibration with $R^2 = 0.97$, $p < 0.001$; Gardarin et al. 2014). Soil C content was measured as described above. SOM was measured by loss on ignition. Fungal and bacterial biomasses were measured by PLFA analysis using the Bligh and Dyer method (1959), as adapted by White et al. (1979) and described by Bardgett et al. (1996). Briefly, this analysis involves the extraction, fractionation and quantification of microbial phospholipids. The fatty acids i150:0, a150:0, 15:0, i16:0, 17:0, i17:0, cy17:0, cis18:1 ω 7 and cy19:0 were chosen to represent bacterial fatty acids, and 18:2 ω 6 was chosen to represent fungal fatty acids (Bardgett and McAlister 1999). Total PLFA content was used as a measure of active microbial biomass. The BFR was calculated by dividing the fungal PLFA marker (18:2 ω 6) by summed bacterial PLFAs (Bardgett et al. 1996); this value was considered to be a proxy for soil mineralisation activity.

Data analysis

Identifying and quantifying ecosystem service bundles poses multiple conceptual and methodological challenges (Mouchet et al. 2014). Consistent with Raudsepp-Hearne et al. (2010), we used a quantitative rather than simply qualitative approach to the analysis of bundles of ecosystem properties, with the aim to cluster grassland plots according to the level of ecosystem services provided and, consequently, in our study to the levels of the seven ecosystem properties used as proxies for ecosystem services. We used multivariate analyses, which make it possible to identify leading correlations among sets of ecosystem services (Lavorel et al. 2011; Maes et al. 2012) and, with advanced multivariate methods, can identify drivers of ecosystem service bundles (Mouchet et al. 2014).

Redundancy analysis and variation partitioning

The matrix of the seven ecosystem properties for each of the 39 plots represented the response variables. Explanatory variables were split into three categories and their corresponding matrices: (1) grassland management (grassland type and date of use); (2) plant functional traits (CWM of each trait); (3) soil properties. Our aim was to quantify the relative contributions of each of these categories to joint variations in the seven ecosystem properties and to identify within each category the most relevant individual variables. For this, we applied an ascending multi-step analysis using multivariate linear regression.

As a first step, to identify variables within each of the three categories significantly associated with variations in ecosystem properties, we ran a forward selection using redundancy analysis (RDA) with either plant functional traits (hereafter 'traits'), soil properties (hereafter

‘soil’; with the three soil-PCA axes as integrated variables) or grassland management (hereafter ‘use’) variables as explanatory variables and ecosystem properties as response variables. In addition to supporting an ascending approach for building statistical models of ecosystem properties depending on plant traits (see, for example, Lavorel et al. 2011), this approach guaranteed that the number of explanatory variables for each step of the analysis remained reasonable related to the number of sampled plots. We applied the two-criteria procedure suggested by Blanchet et al. (2008) to limit the problems of classical forward selections: (1) inflated Type I error was avoided by forward-selecting only models for which all explanatory variables were significant; (2) overestimation of the amount of variance explained was avoided by introducing an additional stopping criterion so that the adjusted coefficient of multiple determination (R^2_{adj}) of the model could not exceed the R^2_{adj} obtained when using all potential explanatory variables. The variables which fulfilled both stopping criteria were identified as the significant traits, soil or use variables influencing the properties of the ecosystems.

As a second step, we used a variance partitioning procedure (Legendre et al. 2005) to quantify the variations in ecosystem properties explained by each category of variables while controlling for the effects of the other categories. The adjusted R^2 (R^2_{adj}) of each of the three models obtained by forward selection, as well as the R^2_{adj} of combined models (traits + soil variables, traits + use variables, soil + use variables, all three groups of variables) were calculated and used to calculate the R^2_{adj} of all the conditional models (traits controlling soil, traits controlling use, soil controlling use, soil controlling traits, use controlling traits, use controlling soil) by subtraction.

Bundling of ecosystem properties

A hierarchical cluster analysis on plot coordinates on the first and second axes of the combined model with all pre-selected management, traits and soil explanatory variables was used to identify bundles (Martín-López et al. 2012).

We used Ward’s linkage method with Euclidean distances to identify relatedness among ecosystem properties (Ward 1963) and automatic selection of the number of clusters as that explaining the largest inertia. The mean levels of ecosystem properties within each bundle were then calculated.

All statistical analyses were performed in R version 2.15.1 (R Core Team 2013) using the library ‘vegan’ 2.0.7, and all variables were standardised, using the function `decostand`, package *vegan* (Oksanen et al. 2013).

Results

Variable selection by RDA and variance partitioning

First, we first present the results of the three successive RDAs used to select variables within each category (use, soil, traits); we then present the final model combining each of these three subsets of variables. Detailed results of the analyses are presented in Table 2. All RDAs were significant at $p < 0.001$.

Akaike information criterion (AIC) scores were similar for the three groups of variables, indicating similar total amounts of variance explained per variable. Among management variables, grassland type (LU) explained 13 % of the variations in ecosystem properties. The inclusion of date of use in the model significantly increased the explained variation to 20 %. The best RDA model for soil variables retained the first two axes of the PCA on soil environmental data as explanatory variables. This model explained 12 % of the variations in ecosystem properties on one single significant axis opposing soil-PCA1 to soil-PCA2, which represented 74.9 % of the total inertia. The best trait-based RDA model explained 18 % of the variations in ecosystem properties and retained VegH, LDMC, LCC and flowering onset as explanatory variables distributed on two significant axes.

Overall, the combined model with these three subsets of variables explained 39 % of the variations in the seven ecosystem properties, with a greater amount of variance

Table 2 Redundancy analysis models explaining variations in ecosystem properties, with selected variables for soil parameters (using the three principal component analysis axes as integrated variables), traits (community-weighted mean value for each trait per plot) and grassland management

Explanatory variables	Akaike information criterion	R^2_{adj} ^a	Model
Use	55.7	0.197	LU + DateUse
Traits	55.8	0.158	VegH + LDMC + LCC + Dateflo
Soil	55.59	0.121	PCA2 + PCA1
Combined	49.46	0.390	LU + DateUse + PCA1 + PCA2 + VegH + LDMC + LCC + Dateflo

LU grassland type, Dateuse date of use, VegH vegetative height, LCC leaf carbon (C) content, LDMC leaf dry matter content, Dateflo mean community date of flowering, PCA1, 2 first and second axis, respectively of the soil principal component analysis (PCA)

^a R^2_{adj} , Adjusted R^2 of the model

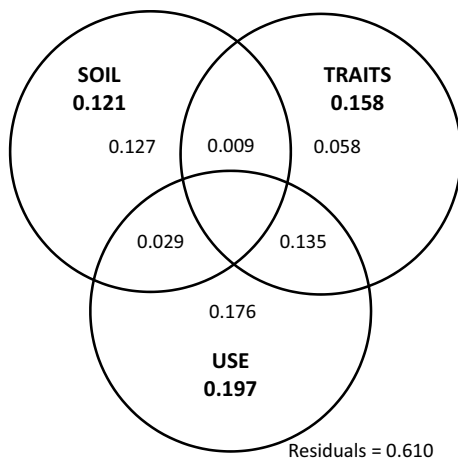


Fig. 1 Venn diagram of the variation partitioning of ecosystem properties explained by grassland management (*USE*), community mean plant traits (*TRAITS*) and soil parameters (*SOIL*). Numbers in bold font are the adjusted R^2 values (R^2_{adj}) of the sub-model (see Table 2). Intersections between two circles represent the variations in ecosystem properties shared in common by both groups of variables (common contribution)

explained per variable than any of the individual models ($\Delta AIC > 2$; Table 2). Management variables showed the highest contribution to the explanation of variations in ecosystem properties (20 %), followed by traits (16 %) and soil parameters (12 %) (Fig. 1). When the contributions shared in common by these three groups (common contributions) were disentangled from the individual contributions of each of the groups, the highest contribution to the total variation in ecosystem properties was found to be the contribution (14 %) shared in common by management variables (*USE* in Fig. 1) and traits (*TRAITS* in Fig. 1), accounting for 44 % of the overall contributions of management variables and traits. The second main contribution was that of soil-alone effects (13 %). Management-alone and traits-alone effects only explained 8 and 6 % of the variations in the seven ecosystem properties, respectively. Lastly, the contribution of soil parameters shared in common with either traits or management variables was very small (1 and 3 %, respectively).

Bundles of ecosystem properties

The clustering of ecosystem properties identified three very distinct bundles (B1, B2, B5) and two intermediate bundles (B3, B4) along the first and second axes of the RDA (Fig. 2, star diagrams). The first axis opposed bundles B5 and to a lesser extent B4 (not significant), both characterised by higher levels of SR and lower levels of all other properties, to bundles B2 and to a lesser extent B1 (not significant), both characterised by lower SR and higher levels of the other ecosystem properties linked with soil or forage (Figs. 2, arrows, 3).

The second axis opposed bundle B2, which maximised the levels of digestibility, ABM and the BFR but showed the lowest levels of soil C content, SOM and TBM, to bundle B1, which maximised these soil ecosystem properties and showed moderate levels of digestibility, ABM and B:F. The intermediate bundle B3 showed rather low levels of all ecosystem properties but maximised digestibility. It is interesting to note that soil ecosystem properties, which were maximised in bundle B1, were orthogonal to SR richness and agronomic ecosystem properties (fodder production and quality), which formed an opposed gradient along which bundles B2–B5 aligned (Fig. 2, arrows).

Taking the explanatory variables (arrows in Fig. 2) into consideration, Bundle B5, which maximised SR, was associated with extensively used grasslands (M5, M6, P5), while bundles B2 and B1, which showed higher levels of agronomic properties, were associated with more intensive uses (M1, P1), with B2 associated with taller plants with lower LDMC and an earlier date of use than other plots. To the contrary, B5 was associated with shorter plants and with higher LDMC and tended to have earlier flowering in plots with a significantly later date of use. B1, which maximised soil C content and SOM, was associated with intensive hay meadows (M1) on cambisols with high scores on soil-PCA1 (high levels of clay and sand, high WHC, lower pH, lower CEC and lower soil N content) and plants with lower LDMC and later flowering.

Discussion

Relative contributions of grassland management and soil and plant traits to covariations among seven ecosystem properties

In this study, we found a dominant effect of grassland management on variations in ecosystem properties. This result is consistent with those of previous studies showing that land uses have long-lasting impacts on ecosystem properties through soil properties and plant communities (Lavorel et al. 2011; Quétier et al. 2007b; Prober et al. 2013). They also corroborate the agronomic grassland typology, which was built a priori on suitability criteria for production systems and included qualitative references to a number of ecosystem properties (ABM, hay digestibility) as well as to some parameters correlated with measured ecosystem properties (e.g. nutrient availability levels, which can be assumed to be related to the BFR; Bardgett and McAlister 1999). Nevertheless, consistent with Lavorel et al. (2011), the inclusion of soil variables and plot mean traits (CWM) increased the explanation of ecosystem properties as compared to models based on management alone. Not surprisingly, soil abiotic variables were mostly associated with

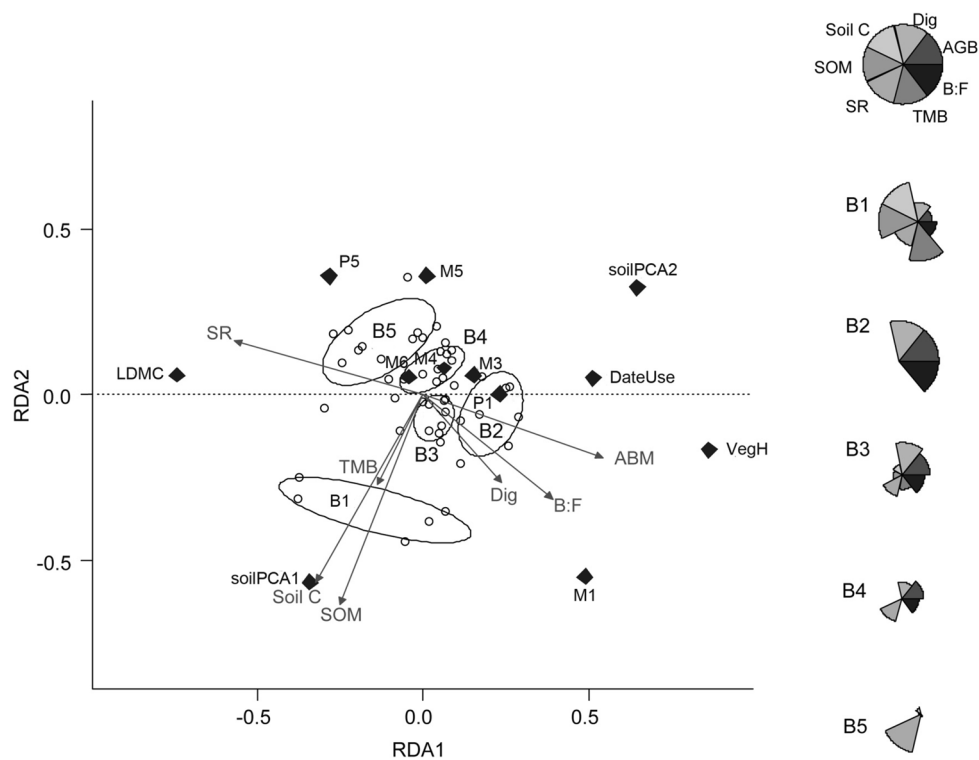


Fig. 2 Bundles (B1–B5) of ecosystem properties identified by the redundancy analysis (RDA) models combining use, trait and soil parameters, followed by a cluster analysis of plot coordinates on RDA axes 1 (horizontal) and 2 (vertical). Star diagrams represent the relative value of the seven ecosystem properties within each bundle (B1–B5) as compared to the maximum across all plots. Ecosystem properties with position in (RDA1, RDA2) plane shown by arrows: ABM Above-ground green biomass, B:F bacteria:fungi ratio, Dig

digestibility, SoilC soil carbon (C) content, SOM soil organic matter, SR species richness, TMB total microbial biomass. Grassland types: M1–M6 Hay meadows, P1 pastured grasslands, DateUse mean community date of flowering, DateUse date of use, LCC leaf C content, LDMC leaf dry matter content, VegH community mean vegetation height, soil PCA1, soil-PCA2 1st and 2nd axes of soil-principal component analyses

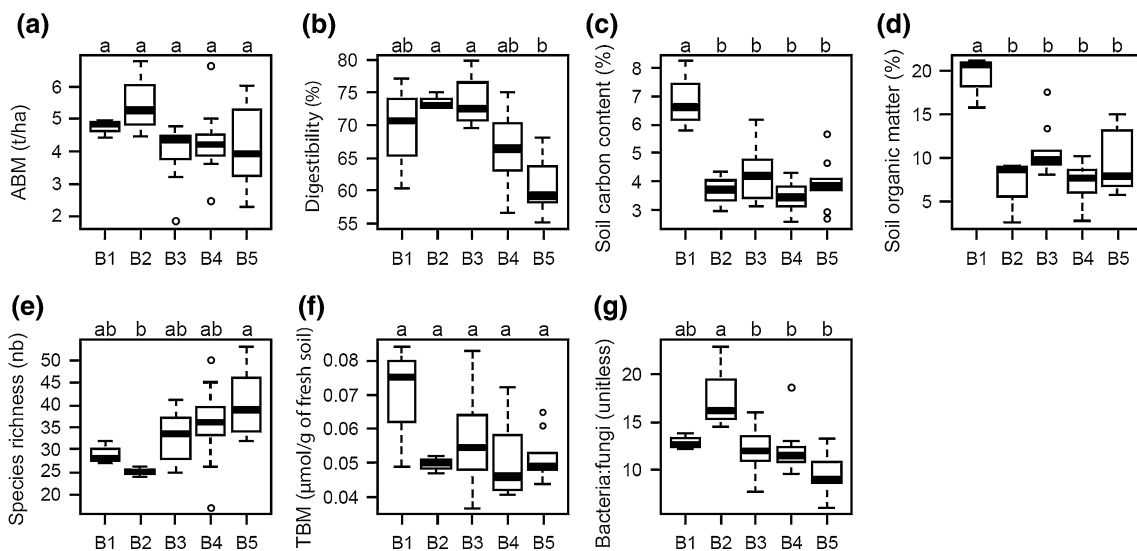


Fig. 3 Boxplots of values for the seven ecosystem properties in the five bundles (B1–B5) identified by cluster analysis on a plot coordinated in the combined RDA. Ecosystem properties: **a** above-ground green biomass (ABM), **b** bacteria:fungi ratio (B:F), **c** digestibility

(Dig) **d** soil C content (SoilC), **e** soil organic matter (SOM), **f** species richness (SR), **g** total microbial biomass (TMB). Different lowercase letters above boxplots indicate significantly different values by Tukey's HSD test

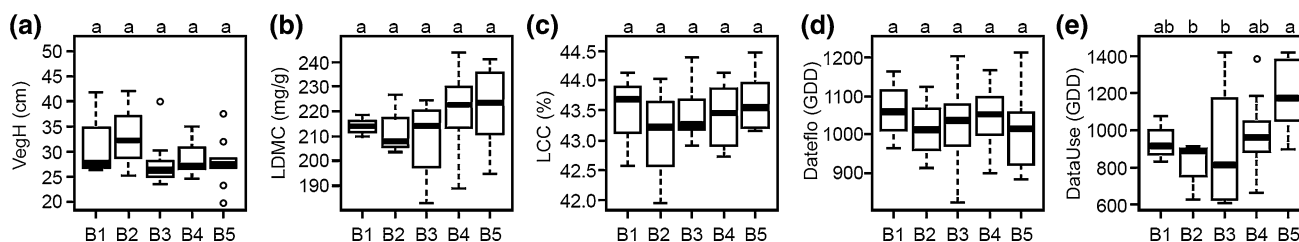


Fig. 4 Boxplots of values of explanatory variables in the five different bundles. **a** Community mean vegetation height (*VegH*), **b** leaf dry matter content (*LDMC*); **c** leaf C concentration (*LCC*), **(d)** mean

community date of flowering (*Dateflo*), **e** date of use (*DateUse*). Different lowercase letters above boxplot indicate significantly different values by Tukey's HSD test

soil-linked ecosystem properties (soil C content, SOM, BFR), while vegetation and management variables were mostly associated with agronomic ecosystem properties (fodder production and quality) and SR. This situation is consistent with plant traits contributing more to above-ground ecosystem properties (e.g. fodder production) and soil biotic parameters contributing more to below-ground ecosystem properties (Grigulis et al. 2013), although Legay et al. (2014) found an equal contribution of soil parameters and plant traits to variations in the BFR ratio in the field. Such results support our hypothesis that below-ground parameters interact with above-ground parameters to determine grassland ecosystem properties and that the relative weights of these parameters depend on ecosystem properties.

The contributions of traits and of grassland management to the observed variations in the seven ecosystem properties overlapped strongly. This result can be explained by taking into consideration previous studies in which vegetative height and leaf traits were shown to be response traits which are not only strongly influenced by grassland management but which simultaneously have significant effects on grassland ecosystem functioning (Lavorel et al. 2011; Laliberté and Tylianakis 2012; Lienin and Kleyer 2012). Moreover, the grassland typology used in our study was based on the physiognomy of dominant graminoids (large, medium or fine-leaved) which is closely linked to leaf traits (in particular, LDMC: Duru et al. 2010).

Overall, the observed variations in the seven ecosystem properties within each grassland type were high, with overall similar coefficients of variation for ecosystem properties across grassland types (Fig. 3; ESM Fig. S1). One-half of the total variance in the ecosystem properties remained unexplained by the RDA (Table 2), suggesting that soil properties and plant traits did not fully succeed in capturing this variation. Recent studies which have considered the contribution of microbial communities in the interaction with soil abiotic parameters and plant traits to ecosystem properties have demonstrated the important effect of microbial communities on the variations in ecosystem properties. For example, Grigulis et al. (2013) showed that SOM

was equally explained by plant traits and microbial traits, and Legay (2013) showed that ABM could be related to all three groups of variables. A part of the remaining variations in our models may thus be partly explained by microbial community traits (Grigulis et al. 2013), as well as by below-ground- rather than above-ground plant traits (Legay et al. 2014). In addition, variation within individual grassland types may reflect the consequences of a variety of past management policies on current management, to which vegetation, and especially soil, may not be fully adjusted (Fig. 4).

Mechanisms underpinning trade-offs among ecosystem properties

The bundling of ecosystem properties highlighted the trade-off between digestibility, the BFR and biomass production (ABM) on the one hand and SR on the other hand (Fig. 2).

The association of LDMC with this gradient confirmed the relevance of the leaf economics spectrum theory to analyses of ecosystem service trade-offs (Lavorel and Grigulis 2012). The leaf economics spectrum describes a gradient from species characterised by less dense and N-rich leaves and fast growth (high specific leaf area, high LNC) to species with denser, nutrient-poor leaves and slower growth (high LDMC, low LNC) (Wright et al. 2004). The link between the functional traits of the leaf economics spectrum and ABM is well supported in the literature (see review by Lavorel et al. 2013). Likewise, digestibility has been shown to be linked with LDMC at the species and community levels (Gardarin et al. 2014), and Grigulis et al. (2013) demonstrated its applicability to a broader set of ecosystem properties associated with nutrient cycling. Finally, exploitative communities (such as type M1 grasslands, with high mean LNC (data not shown) and low mean LDMC, have been shown to be associated with higher BFR (Bardgett and McAlister 1999) and rates of N mineralisation. Fertile habitats, characterised by soil microbial communities dominated by bacteria and rapid microbial activities, have also been linked with greater fodder production, but poor C and N retention (De Vries et al. 2013; Grigulis et al. 2013).

Plot-level SR was overall quite high in this territory, ranging from 17 to 53 species per plot. Individual grasslands contained from 13 to 41 % of the total number of species found on the plateau ($n = 129$). Although there were no significant differences across grassland types, these values represented a gradient of increasing richness from more intensively (M1) to least intensively (M6) managed hay meadows (ESM Fig. S1), which is consistent with previous findings in mid-altitude grasslands (Barbaro et al. 2000; Tasser and Tappeiner 2002; Rudmann-Maurer et al. 2008) and with competitive exclusion by more exploitative species (tall stature, high LNC, low LDMC) under more intensive management (Liancourt et al. 2005).

Perspectives toward ecological intensification

The aim of ecological intensification of agriculture is to increase production based on ecological functions. In our study, we found no significant differences between grassland management types for the NNI (Kruskal-Wallis test, $\chi^2 = 2.47$, $df = 6$, $p = 0.87$), and biomass production (ABM) was associated with neither NNI nor soil N or PNI. This result suggests that nutrients may not be the most limiting factors in this area and that there may be a very narrow potential for any intensification of practices towards greater green biomass production. However, further analysis would need to quantify total annual biomass production, as we analyzed vegetation properties only at the first harvest and thereby missed the finer land use practices linked with the second and sometimes with the third mowing or grazing event.

In contrast, we found that date of use was an important management variable, with significant effects on forage digestibility, a property essential to any intensification of dairy production. Digestibility decreased with later date of mowing (Spearman's rho = -0.54 , $p < 0.05$), consistent with previous findings (see review by Ansquer et al. 2009). It is interesting to note that date of use was somewhat confounded with soil parameters (especially texture and WHC). Intensive hay meadows (M1) and P1 grasslands on cambisols with higher WHC were mown or pastured later and thus showed a lower digestibility at consumption time (data not shown). This illustrates a trade-off between the need to wait until late spring for the soil to drain (for soil protection from trampling) and the dynamics of leaf quality. Later dates of first harvest were also linked with an increase in species richness in mown plots (Spearman's rho = 0.77 , $p < 0.01$), allowing use to reasonably hypothesise a causal link between the delay in date of use and the increase in SR (as well as of other biodiversity indicators, such as the Shannon diversity index; Loucougaray et al. 2015). This implication that the date of use be introduced into such trade-offs among ecosystem properties suggests that, at this site, in

addition to management of fertility, fine choice in practices should be acknowledged in schemes aiming towards ecological intensification. Combining parcels with earlier and later dates of first use across the landscape and within individual farms has been proposed as a strategy to mitigate the primary trade-off between production objectives and biodiversity conservation objectives, as well as to manage risk associated with climate variability (Andrieu et al. 2007).

The RDA analysis showed two well-defined and distinct axes. The first axis opposed fodder properties (ABM and digestibility) and soil nutrient flux (associated with the BFR) to SR, while the second axis was associated with soil stocks (SOM, C and total microbial biomass interpreted as a microbial stock). The first axis was associated with gradients of plant traits and the second axis with soil physical constraints (texture, water availability). This statistical orthogonality between properties relevant to production intensification and soil stocks suggests that plant communities associated with higher fodder quality and quantity may in some cases be compatible with the maintenance of higher soil stocks, an option that should be considered in ecological intensification strategies and for which locations in terms of soils and suitable practices should be devised. For example, grasslands in bundle B3 characterised by high digestibility also supported moderate levels of fertility (BFR) and SOM.

Lastly, our analyses highlight that within a single landscape highly multifunctional grasslands for the chosen set of ecosystem properties, such as those incorporated in B1, coexist with more specialised grasslands, such as those in B2 (production oriented) or B5 (with highest SR values but poor agronomic properties). Ecological as well as the agronomic and social determinants of their distribution will need to be examined. In addition, further analyses may incorporate weighting coefficients according to preferences by different stakeholder groups or policy aims so as to highlight corresponding social trade-offs and their impacts on management prioritisation (Gos and Lavorel 2012).

Conclusion

Overall, our analyses confirm joint effects of management, traits and soil abiotic parameters on the variations in the ecosystem properties of grasslands, with a predominance of management and traits. These drivers had overlapping effects on the observed variations in ecosystem properties. The variations explained by traits were consistent with the leaf economics spectrum model and its implications for ecosystem functioning. The observed independence between ecosystem properties relevant to production (forage biomass, digestibility and nutrient turnover) on the one hand and soil stocks (SOM, C and microbial stocks) on the other hand suggests that an intensification of fodder

production might be compatible with the preservation of the soil resource base on suitable soils and with future practices yet to be devised. We highlighted that fine choices of practices, such as the first date of grazing or mowing depending on soil moisture, have important consequences on various ecosystem properties relevant to ecosystem services and may influence biodiversity patterns. Such avenues for ecological intensification should be considered as part of further landscape- and farm-scale analyses of the relationships between farm functioning and ecosystem services.

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Author contribution statement SL conceived and supervised the study; SL, GL and PG designed the field campaign; MPC coordinated the field campaign and data management; all authors participated the field work. CA was responsible for lab analyses of soil and plant material; PG and SL analysed the data and led the writing of the manuscript, with contributions from GL and SG.

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