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Predicting forage quality of species-rich pasture grasslands using vis-NIRS to reveal effects of management intensity and climate change

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ABSTRACT

With a growing human population facing multiple global change drivers (i.e. climate change and land management change), the future of food security is of major importance. Sustainable agriculture is therefore key to ensure food supply and food security under future climatic conditions. Forage provision (composed of forage quantity and forage quality) is an important ecosystem service of grasslands for dairy production. However, monitoring forage quality in semi-natural species-rich grasslands is rarely done due to the inherent complexity in determining forage quality, high variability within natural systems and financial and workload restrictions. Here, we i) demonstrate the ability of visible-near-infrared spectroscopy (vis-NIRS) to predict forage quality of bulk samples of species-rich montane pastures and ii) show its potential to reveal effects of two key global change drivers, climate change and land management, on forage quality. Spectral information and chemometrics allowed us to predict three (ash, fat and protein) out of four analyzed forage quality parameters with high accuracy. Land management intensity strongly influenced species-rich grasslands' protein and fat content, whereas altered climatic conditions influenced ash and fat content. High management intensity increased protein content of high- and mid-elevation pastures by 22 % and 30 % and fat content by 19 % and 20 % respectively. Though forage quality was improved by intensive land management, extensive land management generally revealed sufficient forage quality for livestock. Vis-NIRS provides a rapid, cost-efficient and high-throughput technique to analyze forage quality, revealing effects of global change drivers on forage quality of grasslands. This approach will help to support stakeholders assure optimal nutrition feeding of livestock and achieve steps towards sustainable agriculture.

1. Introduction

Food demand is increasing with increasing human population (FAO, 2013; Godfray et al., 2010), however, land area suitable for agricultural production is limited (Azar, 2005; Stoll-Kleemann and O'Riordan, 2015; West et al., 2014) and intensification is unlikely to provide sustainable solutions (Allan et al., 2015; Foley et al., 2005; Gossner et al., 2016; Laliberté et al., 2010). Thus, to meet future human food demands, agriculture must sustainably increase production from less land through efficient use of natural resources and with lowest impact on the environment (Hobbs et al., 2008). Forage provision for cattle and dairy is a major ecosystem service of agricultural grasslands. Forage provision determines carrying capacity and performance of livestock (Bailey et al., 1996; Schauer et al., 2005) and consists of two parts: quantity (yield or production) and quality (the nutritional value for livestock) (Beeri et al., 2007). Ongoing climatic changes as well as changes in land management intensity are likely to change both components of forage provision (Martin et al., 2014). While forage quantity is straightforward to assess, gaining measures of forage quality (i.e. crude protein, crude fat, crude fiber and crude ash) is challenging due to being multivariate and costly in time and money. Nonetheless, forage quality is equally important for maintaining a sufficient supply of energy and nutrients

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for livestock from plants. Failure to mitigate and adapt agriculture to changing environmental conditions can reduce economic value of grasslands and can even be a threat to food security (Richards et al., 2019). To maintain ecologic and economic values of grasslands under future changing conditions, we need to understand the effects of climate and land management on forage quality, which requires an effective and rapid tool to monitor forage quality.

Forage quality of grasslands increases with increasing protein and fat content, but decreases with increasing fiber content (Deak et al., 2007; Li et al., 2018; Xu et al., 2018). Forage quality is affected by species composition and abundance (Khalsa et al., 2012) and soil resource availability (Niu et al., 2016), which are both in turn affected by climatic conditions and land-use intensity. Land-use intensification ultimately reduces ecosystem stability (Blüthgen et al., 2016) by homogenization of landscapes (Gossner et al., 2016) and loss of biodiversity (Allan et al., 2015; Flynn et al., 2009; Newbold et al., 2015). Further, chemical, physical and biological soil properties, such as nutrient mining, compaction (Smith et al., 2016) and soil biodiversity (Tsiafouli et al., 2015) are negatively affected by land-use intensity. Climate change decreases soil organic carbon (Puissant et al., 2017), increases gross nitrogen turnover (Wang et al., 2016), and causes shifts in both aboveground plant communities (Gornish and Tylianakis, 2013; Klanderud and Totland, 2005) and belowground soil communities (Blankinship et al., 2011). Consequently, climate change and land management can interact both synergistic and antagonistic (Hasibeder et al., 2015; Ingrisch et al., 2017; Karlowsky et al., 2018).

In grassland systems, there is a need for increased research and monitoring of forage quality under global change scenarios. Rapid progress during the last decades in physics and engineering has opened a variety of optical measurements, notably near-infrared spectroscopy (NIRS) and closely related techniques such as vis-NIRS (visible near-infrared spectroscopy). The wavelengths covered by vis-NIRS is extended at the lower range down to 350 nm in comparison to the lower boundary of NIRS (800 nm), thus offers a broader spectral range to detect organic complex molecules. In comparison to the wet-chemical standard analysis (i.e. improved and adjusted Weender-analytic), which is time-consuming, expensive and demands relatively high sample masses, NIRS is a cheap and fast high-throughput technique. This opens the opportunity for quick and efficient analysis of large sample numbers (Foley et al., 1998; Osborne et al., 1993; Shenk and Westerhaus, 1993). NIRS is a qualitative and quantitative analysis technique. It is based on the absorption and reflectance of the analyzed sample. The chemical composites of a sample (e.g. element, chemical bond, chemical structure) get excited by NIR radiation each to a specific degree, in turn reflecting a unique proportion of radiation for wavelengths and its molecular overtones (Bokobza, 2002). In organic substances in particular chemical bonds (such as CH, CC, C=C, CN, OH, NH) help to identify macromolecules of high complexity (Ludwig and Khanna, 2001). However, NIRS always needs to be calibrated to a chemical analyzed subset of samples. The process of predicting the parameter of interest for unknown samples requires development of multivariate models with accompanying quality checks. A further key in the successful application of NIR spectrometry lies in proper processing of spectral data and model validation to extract the appropriate information from the spectra (Gautam et al., 2015; Liland et al., 2016; Morais et al., 2019; Ng et al., 2018). After model development, NIRS allows for relatively rapid identification and quantification of several parameters of interest from a plant samples' spectra. Thus, with NIRS we can extrapolate the parameters of interest for a large set of samples out of a considerably smaller subset of samples (Foley et al., 1998; Lawler et al., 2006).

NIRS has advanced in its application in basic and applied science (Ozaki, 2012). In vegetation ecology, NIRS has been implemented in elemental stoichiometry, non-structural carbohydrate or forage quality analysis of e.g. aquatic grasses (Lawler et al., 2006), terrestrial plant functional types (Anderson et al., 2018) and single species (Murguzur et al., 2019; Quentin et al., 2016), dry matter yield of mixed sown communities (Biewer et al., 2009), different plant organs (Ramirez et al., 2015), remote sensing techniques to estimate forage provision (Beeri et al., 2007) or even classify or identify communities and species (Durgante et al., 2013; Richter et al., 2016; Ustin and Gamon, 2010). Hitherto, the application of NIRS on forage quality of bulk samples from species-rich grasslands is scarce (Fekadu et al., 2010; Parrini et al., 2018) and to our knowledge has not been used to reveal effects of multiple global change drivers on forage quality. To be able to meet the goals of sustainable agriculture under future environmental and demographic conditions, we need to be able to rapidly track forage quality and understand the interplay between major global change drivers and their effects on forage quality.

The goal of this study is twofold. First, we test the ability and accuracy of vis-NIRS to predict classic forage quality (i.e. crude ash, crude fat, crude fiber and crude protein as determined from wet-chemical analysis) of mixed bulk samples of central European species-rich, semi-natural, montane grasslands. Second, we demonstrate the application of vis-NIRS by testing the influence of two major global change drivers, climate change and land management intensity, on forage quality of such grasslands. Our ultimate aims are to inform and improve grassland management with a flexible and readily accessible tool to measure and monitor forage quality of agricultural lands in the face of changing conditions.

2. Material and methods

2.1. Sample collection

Samples were collected within the land-use experiment of the Project: "Sustainable use of alpine and pre-alpine grassland soils in a changing climate" (SUSALPS). In 2016, intact plant-soil mesocosms of two extensively managed, montane grasslands Esterberg and Graswang were translocated downslope along an elevational gradient in the TERENO Pre-Alpine Observatory in order to simulate climate change (increased temperature and reduced precipitation). The elevational gradient of translocation ranges from 1260 m a.s.l. (Esterberg) via 860 m a.s.l. (Graswang) to 600 m a.s.l. (Fendt). In 2017 and 2018, the years of study, this elevational gradient represented the two year mean annual temperatures and mean annual precipitations of 6.3 °C, 1113 mm at high elevation (Esterberg), 7 °C, 1433 mm at mid-elevation (Graswang) and 9 °C, 1036 mm at low elevation (Fendt). Thus, a warming of +1 K from high- to mid-elevation, +2 K from mid- to low-elevation and consequently +3 K from high- to low-elevation. We focus on altered temperature and precipitation as they are likely to exert the strongest effect on plant physiology. However, we acknowledge potentially other climatic changes to co-occur by downslope translocation, but believe that these factors likely only have a minor impact on plant communities in this study given the limited geographic extent of translocation. Communities from Esterberg and Graswang were translocated downslope to lower elevational levels as well as reburied at the site of origin as controls. Translocation and reinstallation procedure is described in Berauer et al. (2019). For an overview of experimental site descriptions see Supplementary Table 1, additional climatic conditions are displayed in Supplementary Fig. 1. Each of the communities was assigned to either an intensive or extensive land management intensity ($n = 6$ for each origin x recipient x land management combination) leading to 60 translocated communities in total. Intensive managed communities were cut (3 cm aboveground; simulating grazing or mowing) and fertilized (slurry application; with nitrogen input equivalent to 42 ± 10 kg N ha^{-1} per fertilization event) 5 times a year, whereas extensive managed communities were cut 3 times and received slurry only after the first and last cut within each growing season. These applied management regimes were selected because they are common practices within this region and elevational range and thus represent realistic agricultural practices for future grasslands. Community bulk samples of aboveground biomass were collected according to the local farmers' practice within the growing season of each year. From 2016 until 2018, a total of 515 bulk samples of montane grassland communities were collected and used here for analysis. For a detailed overview of dates of land-use management application see Supplementary Table 2.

2.2. Sample preparation

All collected samples were dried at 60 °C for 48 h and weighed. Samples were then first homogenized at 2 mm using a shredder (SK1, Retsch GmbH, Germany) and subsequently milled to powder using a ball-mill (MM301, Retsch GmbH, Germany). With this procedure, we obtained a homogenous and representative mixture of semi-natural, species-rich grassland samples. For each sample, a minimum of 3.5 ml volume of powder was milled and filled to an Eppendorf tube.

2.3. Spectral measurement

All spectra were measured in the darkroom laboratory using a vis-NIR analyzer (SVC HR 1024-i, Spectra Vista Corporation, USA) with a 4° fore optic lens. Spectral range was 350 nm – 2500 nm with a spectral resolution of 3.3 (at 700 nm), 9.5 (at 1500 nm) and 6.5 (at 2100 nm). To be able to produce representative and repeatable spectra, an external source of light (VNIR Light Source, Hyspex, Norway) was used and installed to avoid any shade from either the equipment set-up or the sample material. For the spectral scan, previously milled bulk sample material (each representing a mesocosm with 30 cm diameter) was filled into a plastic petri-dish (3 cm diameter) covering the bottom up to at least 0.5 cm and subsequently scanned 3 times at random positions. Each spectra was corrected for the offset between the three detectors, as described in Kühnel and Bogner (2017) and then smoothed by the singular spectrum analysis (SSA) with a window length of 50 using the package *Rssa* of the software R (Golyandina et al., 2013; Golyandina and Korobeynikov, 2014; Korobeynikov, 2009). Subsequently, the three spectra per sample were averaged. Three of the 515 samples had to be removed due to insufficient quality of the spectral data.

2.4. Wet chemical analysis

Wet chemical analyses of forage quality were conducted following standardized Weender-analytics (SGS Germany GmbH, Hamburg, Germany). A subset of 70 samples was chosen based on NIR spectral data for cross referencing using the Kennard-Stone algorithm with an Euclidean distance metric (Kennard and Stone, 1969), which selects representative samples to systematically cover the spectral variance in all samples. The selected samples are a representative mixture of all experimental combinations (origin, transplant, treatment and year). The algorithm had a total of 336 samples to choose from, as prior to calculations, samples gathered in the year of experimental set-up as well as samples with less than 15 g of biomass had to be removed. This relatively high amount of biomass provides a minimum measure quantity for the analyzed parameters of forage quality: crude ash, crude fat, crude fiber and crude protein.

2.5. Statistical analysis

2.5.1. Chemometric: vis-NIRS model for prediction of forage quality

Partial-Least-Square regression (PLS) was applied to predict forage quality (ash, fat, fiber, protein) of species-rich grasslands' tissue samples. We used the entire measured spectra ranging from 350 nm to 2500 nm. First derivate of spectral data was calculated prior to calibration-modeling, as it improved model performance in comparison to raw, vector-normalized or first derivate of vector-normalized spectra.

For model development, we split the available samples (n = 70) into a calibration set (n = 50) and an external validation set (n = 20). For this split, we conducted Kennard-Stone algorithm, again systematically selecting samples to represent the maximum spectral variance and to mimic the initial sample selection. A calibration-model was built using Partial-Least-Squareregression (PLS) in combination with a variable selection procedure (CARS; competitive adapted reweighted sampling) to obtain the most parsimonious and robust models (Li et al., 2009). The Monte Carlo based approach CARS selects for an optimum number of wavelength with root mean squared error (RMSE) and an optimum number of latent vectors by leave-one-out cross-validation (Li et al., 2009). We used 50 CARS iterations, repeated 100 times to identify the best model with the lowest RMSE in cross-validation. For the simulation, a maximum of 10 latent vectors was set. Model performance was evaluated by the coefficient of determination (R^2), root-mean-squared error (RMSE) and residual prediction deviation (RPD; ratio between standard deviation of the prediction to standard error).

2.5.2. Application: effect of climate change and land management intensity on forage quality

To test for effects of climate change and land management on forage quality, first parameters of forage quality suitable for prediction via vis-NIRS were identified; then all 512 gathered samples were predicted using the best model selected from CARS-PLS. Subsequently, we used linear mixed effect models to test the effects of treatments on individual fodder quality parameters, always including Plot-ID as a random factor to account for non-independence of samples by repeated measurements. First, we analyzed the data of 2017 and 2018 separately with land-use management, climate change as fixed factors and the interaction between both global change drivers. Secondly, where ANOVA revealed significant effects of model terms with multiple comparisons, we performed a Tukey HSD post-hoc test. For all models conformity of model assumptions (normal distribution and homoscedasticity of variances) were checked.

We calculated effect sizes to report on relative differences between either land-use management or climate change effects on parameters of forage quality. For effects of land management intensity, we used extensively managed communities (with low cutting and fertilization frequency as described in 'Sample collection') and for climate change effects we used the on-site of origin installed controls as reference.

All statistical analysis were performed in R Version 3.5.3 "Great Truth" (R Core Group, 2019) using the packages *pls* (Mevik et al., 2019), *nlme* (Pinheiro et al., 2019), *lsmeans* (Lenth, 2018).

3. Results

3.1. Predicting forage quality of species-rich grasslands (bulk samples)

Three out of four forage quality parameters were identified as suitable for measurements with vis-NIRS, namely ash, fat and protein, but not fiber. With CARS-PLS we identified robust and parsimonious models with high accuracy and predictive power in both internal (cross) as well as in external validation (see Fig. 1A and B). The selected wavelengths and their relative importance to model performance are shown in Fig. 1C.

Across all four modeled parameters, the best model was obtained for protein with the model evaluation of external validation (R^2_{val} = 0.83; RPD_{val} = 2.4; $RMSE_{val}$ = 1.06) and of calibration (R^2_{cal} = 0.93; RPD_{cal} = 3.76; $RMSE_{cal}$ = 0.47). The model consisted of 8 latent vectors built upon 93 selected wavelengths, which corresponds to 4.3 % of the 2150 wavelengths within the spectra. The second highest accuracy was achieved for communities' ash content with model evaluations of external validation (R^2_{val} = 0.71; RPD_{val} = 1.87; $RMSE_{val}$ = 0.79) and calibration (R^2_{cal} = 0.83; RPD_{cal} = 2.42; $RMSE_{cal}$ = 0.47). The 9 latent vectors building this model used 29 wavelengths, corresponding to only 1.3 % of the spectra. For fat content model evaluation of validation (R^2_{val} = 0.73; RPD_{val} = 1.69; $RMSE_{val}$ = 0.37) and calibration (R^2_{cal} = 0.86; RPD_{cal} = 2.65; $RMSE_{cal}$ = 0.15) is sufficient. To build this model

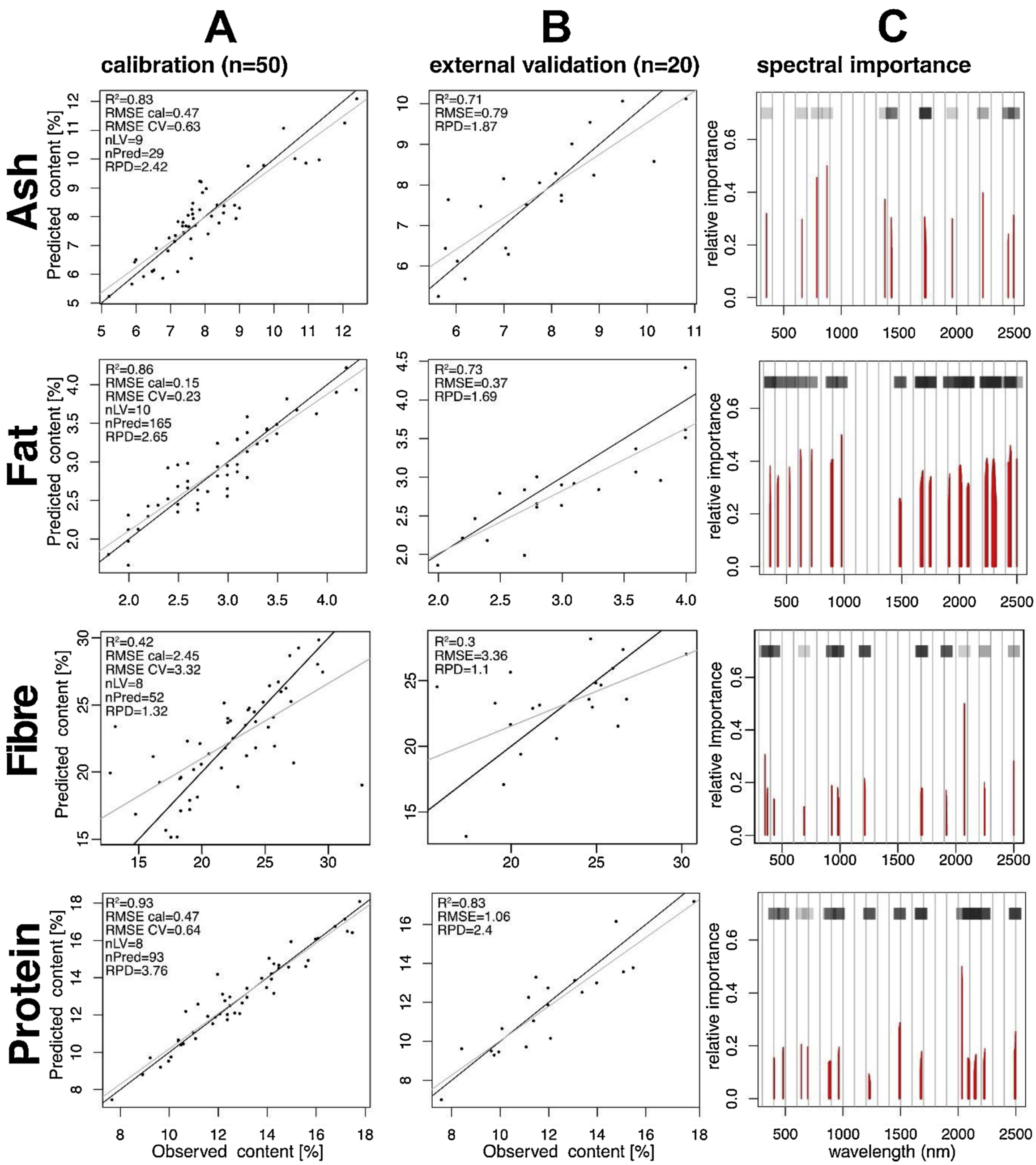

Fig. 1. Calibration (A, left panel), external validation (B, center panel) and importance of spectral regions (C, right panel) for parameters of forage quality of the selected best CARS-PLS model. Model descriptions and quality estimates are given in the top left corner of each respective panel. With R^2: coefficient of variation of the respective model; RMSE cal: root mean square error of calibration model; RMSE CV: root mean square error of internal cross-validation; nLV: number of latent vectors; nPred: number of predicting wavelengths; RPD: residual prediction deviation.

10 latent vectors of 165 wavelengths were build. These selected wavelengths represent 7.7 % of the entire spectra. For fiber we achieved insufficient model evaluations for prediction, both validation (R^2_{val} = 0.3; RPD_{val} = 1.1; $RMSE_{val}$ = 3.36) and calibration (R^2_{cal} = 0.42; RPD_{cal} = 1.32; $RMSE_{cal}$ = 2.45) indicate a high predictive error and inaccuracy.

For protein, ash and fat the RPD in combination with the respective R^2 indicate “good” to “excellent” predictions, whereas the calibration for fiber is not usable (Malley et al., 2004; Saeys et al., 2005). Nonetheless, please note the deviation in model criteria between calibration and external validation for all three parameters, especially fat content.

For a detailed summary of model selection, estimates and quality criteria see Table 1.

The wet chemical determined percent concentration of dry matter of the forage quality parameters ranged between 5.24 and 12.42 (ash), 1.8 and 4.3 (fat), 12.8 and 32.5 (fiber) and 7.6 and 17.8 (protein) in the wet-chemical analyzed sub-set of samples. The range of the entire forecasted spectral dataset of the three parameters identified for robust prediction is 2.06 and 14.47 (ash), 0.72 and 5.31 (fat) and 5.95 and 28.70 (protein).

3.2. Effect of climate change and land management intensity on forage quality

Both climate change (increased temperature and altered precipitation) and land management intensities (different cutting and

Table 1
Description of CARS-PLS model performance and quality estimates of the selected best model. All models were built on first-derivate of spectra with allowed maximum latent vectors of 10. Dataset was split into calibration and external validation using the Kennard-Stone algorithm.

		Ash	Fat	Fibre	Protein
	# latent Vectors	9	10	8	8
	# Predicting Wavelength	29	165	52	93
R^2	*calibration*	0.83	0.86	0.42	0.93
	external validation	0.71	0.73	0.3	0.83
RPD	*calibration*	2.42	2.65	1.32	3.76
	external validation	1.87	1.69	1.1	2.4
RMSE	*calibration*	0.47	0.15	2.45	0.47
	internal validation	0.63	0.23	3.32	0.64
	external validation	0.79	0.37	3.36	1.06

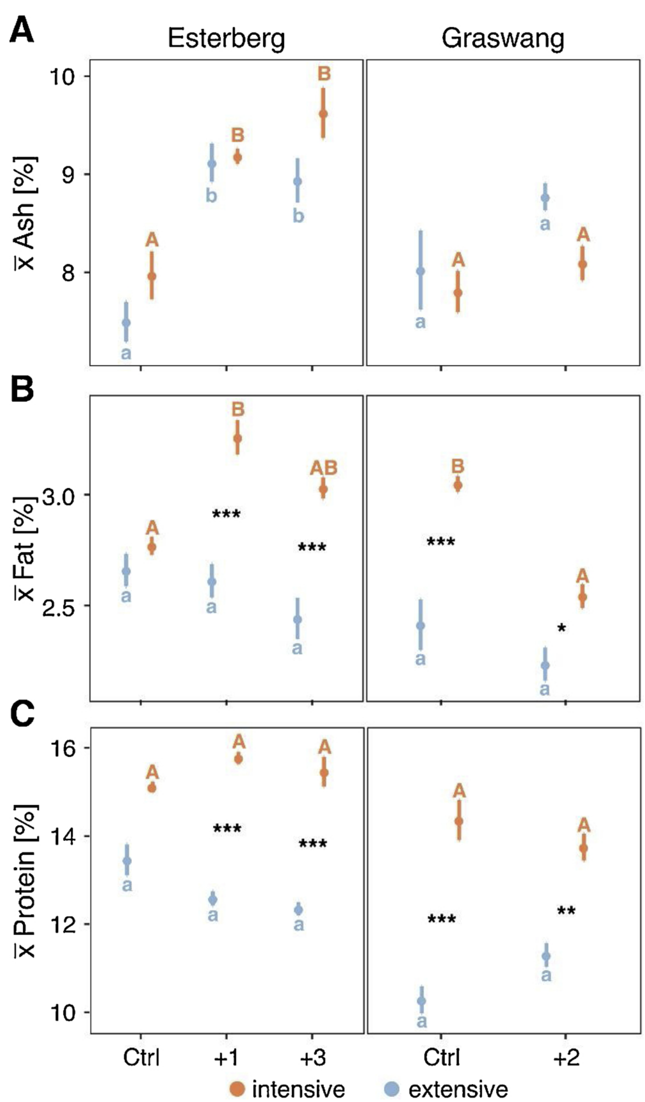

Fig. 2. Effects of land-use management and climate warming (x-axis) on forage quality in the year 2017 of both translocated communities. Shown are arithmetic mean and standard error of each parameter predicted with CARS-PLS. Asterisks indicate differences between land-use management within each climatic site (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) and letters (colored according to land-use regime; additional upper and lower case for separation) indicate significant difference between climatic sites within each land-use regime. p-values of ANOVA and according TukeyHSD post-hoc test are displayed in Supplementary Table 6 and Supplementary Table 7.

fertilization frequency) influenced community forage quality using the full set of 512 samples. Here, we describe in detail the results of the year 2017 (Fig. 2). Similar analyses were also conducted for the data gathered in 2018. Results and summaries of analysis as well as effect size of both years can be found in the Supplementary Material (Supplementary Tables 3 – 7; Supplementary Fig. 2).

In general, intensive (five cuts and five slurry applications per growing season) land management increased both fat and protein content relative to extensively (three cuts and two slurry applications per growing season) managed communities originating from high-elevation Esterberg (19.4 % increase for fat and 22 % increase for protein) and mid-elevation Graswang (20.1 % increase for fat and 30.3 % increase for protein). Land management showed opposing trends on communities' ash content, with an increase of 5 % for intensive communities from high-elevation Esterberg and a decrease by −5.4 % for intensive communities from mid-elevation Graswang compared to extensive communities. Climate change effects from warmer and drier conditions were highly variable across forage quality parameters and depended on the origin of communities (see Supplementary Table 3 for an overview of relative change by either land-use management or climate change).

3.2.1. Effect of management intensity on forage quality

Land management intensity affected fat ($p_{Graswang} < 0.001$; $p_{Esterberg} < 0.001$) and protein ($p_{Graswang} < 0.001$; $p_{Esterberg} < 0.001$) content of both translocated communities. Further the interaction between land-use intensity and climate change effected fat content ($p_{Esterberg} < 0.05$) but only for communities from high-elevation Esterberg.

Intensive management in comparison to extensive management increased both fat (26.1 %; $p < 0.001$) and protein (39.7 %; $p < 0.001$) content of communities from mid-elevation at their site of control. Similarly to their site of control, fat (13.7 %; $p < 0.05$) and protein (21.7 %; $p < 0.01$) content of communities from mid-elevation Graswang increased by intensive management under altered (+2 K) environmental conditions (see Fig. 2B and C, the right panel).

Communities from high-elevation Esterberg did not show any significant difference introduced by land management intensity at their high-elevation control site. Yet, under altered environmental conditions after translocation to mid-elevation (+1 K warming) and low-elevation (+3 K) protein ($p_{+1K} < 0.001$; $p_{+3K} < 0.001$) and fat ($p_{+1K} < 0.001$; $p_{+3K} < 0.001$) content of communities originating from high-elevation Esterberg increased by intensive management. At +1 K warmed conditions protein content increased by 25.4 % and at +3 K by 25.2 %. Similar, fat content of high-elevation communities increased by 24.6 % at +1 K and 24 % at +3 K warmer conditions (see Fig. 2B and C, the left panel).

3.2.2. Effect of climate change on forage quality

Climate change affected ash content, but only in communities originating from high-elevation Esterberg ($p_{Esterberg} < 0.001$). Whereas, fat content of communities originating from both sites was influenced by climate change ($p_{Esterberg} < 0.01$; $p_{Graswang} < 0.001$).

Ash content increased with warming irrespective of land management intensity at both +1 K ($p_{INT} < 0.05$; $p_{EXT} < 0.05$) and +3 K ($p_{INT} < 0.01$; $p_{EXT} < 0.05$) levels, but no difference between +1 K and +3 K warming was found for communities originating from high-elevation Esterberg.

Fat content of intensively managed communities originating from high-elevation Esterberg was 17.6 % higher at +1 K warming in comparison to the intensively managed controls ($p < 0.001$). Contrasting to this, fat content of intensive managed communities of mid-elevation Graswang *decreased* by 16.5 % ($p < 0.001$) under warmer conditions in comparison to the similar land management intensity at the control site.

4. Discussion

Our results showed that visible-near-infrared spectroscopy (vis-NIRS) is a feasible tool to quantify forage quality in species rich communities undergoing structural shifts due to global change drivers. With CARS-PLS we identified robust, parsimonious and accurate models for ash, fat and protein but not for fiber content of communities' bulk samples. Applying these techniques to our experimental plant communities revealed strong and clearly differentiable effects of climate change – increasing temperature and decreasing precipitation – and land management regime – high versus low mowing and slurry application frequencies - on forage quality. Protein content was increased by higher mowing frequency and slurry application (Aavola and Kärner, 2008; Pavlů et al., 2011), but not affected by climate change, adding further evidence to inconsistent effects of warming on protein content (Dumont et al., 2015; Xu et al., 2018). Ash content varied with increases in temperature and decreases in precipitation, and fat content was interactively affected by both global change drivers (Grant et al., 2014; Li et al., 2018). vis-NIRS offers a high potential for monitoring and predicting changes in forage quality under changing environments (Anderson et al., 2018; Murguzur et al., 2019; Parrini et al., 2018).

4.1. Predicting forage quality using vis-NIRS analysis

All three parameters with robust and parsimonious have a low RMSE of external cross validation. The RMSE for protein, ash and fat represents 4.7 %, 6.4 % and 8.1 % of the entire range spanned within all predicted samples. According to the criteria introduced by Saeys et al. (2005), our calibration range from "good" for fat and ash (on the brink) to "excellent" for protein. These good calibration model of protein is caused by the spectral relevance of nitrogen linked to the adsorption NH bond (Roberts et al., 2004), which is a major component of proteins, and the larger range of protein in our samples in comparison to ash or fat. This high accuracy predictions of forage quality are aligned with different studies of standardized sown communities such as alfalfa (De Boever et al., 1998), wheat (Cozzolino et al., 2006), ryegrass (Jafari et al., 2003; Smith et al., 2019), sown mixtures of varying complexity (Deak et al., 2007), as well as species-rich natural meadows (Sonia Andrés et al., 2005; Danieli et al., 2004; Fekadu et al., 2010; Parrini et al., 2018).

The wavelengths selected by CARS-PLS for all of the analyzed parameters of forage quality are within the region typical for organic molecules and the chemical bonds characterizing organic macro molecules of higher complexity (Kawamura et al., 2008; Ludwig and Khanna, 2001).

However, with respect to fiber content our approach did not lead to a model with high predictive power and accuracy. Studies of both sown, species-poor plant communities (Sanz-Sáez et al., 2012) and semi-natural, species-rich plant communities (Danieli et al., 2004; Parrini et al., 2018) successfully used NIRS to predict fibrous content, e.g. acid detergent fiber, neutral detergent fiber or crude fiber. Among the fibrous parameters, crude fiber was the one with the weakest model performance found by Danieli et al. (2004) and Parrini et al. (2018) in natural grasslands, but still remarkably higher than in our results. Despite the range of crude fiber in here is comparable to those reported in Danieli et al. (2004) and Parrini et al. (2018). We can only speculate, but this result might be based on the primary wet-chemical (Weender analytics) which allows only an approximate estimation of crude fiber, thus the follow-up prediction by vis-NIRS only propagates this uncertainty (S. S. Andrés et al., 2005; Sonia Andrés et al., 2005; Roberts et al., 2004). Spectral information is unlikely to cause the failure here, as wavelengths critical to determine fiber (characteristic chemical bonds CH and OH) are within our investigated spectral region (Kawamura et al., 2008).

4.2. Effects of climate change and land management regime on forage quality

We detected effects of both global change drivers - climate change and land-use intensity – on forage quality. The effects of climate change on ash and fat content varied depending on land management and origin of community. Effects of warming on forage quality in mountain regions is sparse and so far did not reveal a consistent significant response to warming (Dumont et al., 2015). Yet, previous experimental studies on the Tibetan high plateau found an increase in fat and protein content and a decrease of parts of the fibrous fraction with warming but only under dry conditions (Li et al., 2018; Xu et al., 2018). This positive effect of warming on forage quality was mediated by changes in community composition induced by altered environmental conditions (Li et al., 2018; Xu et al., 2018) and was shown to also hold true on large geographical scales (Shi et al., 2013). The high variability in communities' response to climate change in our study may hint towards an indirect effect of climate change mediated by community composition, although we lack the data to test this. Cold adapted and montane communities are expected to shift towards more thermophilic species (Gottfried et al., 2012; Rumpf et al., 2018), higher graminoid abundance (Klanderud et al., 2015; Winkler et al., 2016) and are more susceptible to novel competitors (Alexander et al., 2015). Our translocated communities were only exposed to novel climatic conditions for two years. Yet, the early stages of changes in community composition by altered climatic conditions of the same grasslands without management showed non-deterministic changes in community composition change (Berauer et al., 2019). These effects on biodiversity are likely to be amplified by land management intensity, namely fertilization and cutting (Socher et al., 2013).

Land-use intensity increased forage quality by increasing fat and protein content of communities from both origins, thus improving forage quality in general. This effect was non-significant for the high elevation control of communities in Esterberg. We attribute this exception to the especially short growing season in 2017, which is also reflected in the land-use intensity in 2017 of this experimental site (see Supplementary Table 2). In 2018, protein content of high-elevation Esterberg control communities increased with land management intensity, further supporting our argument (see Supplementary Fig. 2)

Higher cutting frequency can increase leaf nitrogen and protein content (Pavlů et al., 2011; Walter et al., 2012; White et al., 2014) in comparison to lower cutting frequency. Conversely, reduction or cessation in cutting was shown to increase leaf dry matter content and reduce forage quality (Lavorel et al., 2011). Fertilization also increases forage quality via leaf nitrogen status in grasslands (Aavola and Kärner, 2008; Liu et al., 2010; Malhi et al., 2010). This matches our findings here of increased protein content (which incorporates the largest fraction of nitrogen in the organic molecules we tested). Whithead (2000) recommends a protein content ranging from 12 to 19 % to meet dairy cattles' requirements. Protein content of all intensively managed and most extensively managed communities are above this recommended threshold and only the protein content of extensive managed communities originating from mid-elevation Graswang are lower ($Graswang_{ctrl}$ = 10.3 %; $Graswang_{+2K}$ = 11.3 %). The amount of gaseous nitrogen-oxides loss immediately after slurry application of similar grasslands (exposed to similar climate change and land-use intensity) was recently found to be higher than previously assumed rates of gaseous N losses (Zistl-Schlingmann et al., 2019). This would likely reduce the amount of plant available nitrogen added by slurry application. With the possibility of "real-time" monitoring of forage quality, the timing and amount of fertilizer application could be adapted, which is key for sustainable grassland management (Wohlgemuth et al., 2019).

The lack of interaction between both manipulated global change drivers on forage quality indicate that both climate change and land management intensity are acting with independent mechanisms on forage quality, at least in the short-term.

Forage quality of species-rich communities is highly variable and can be influenced by species composition and abundance (Khalsa et al., 2012), nutrient availability (White et al., 2004) or stage of maturity (Waramit et al., 2011). Despite this natural variability, monitoring forage quality and adapting land-use regimes as needed remains an important task for sustainable agriculture under future conditions. Our study is the first that we know of to use NIRS to track forage quality in managed grasslands undergoing rapid change in response to global change drivers. Given the future threats these drivers pose to these grasslands' biodiversity and ecosystem function, such tools and monitoring schemes will be essential for maintaining sustainable agriculture under future conditions.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.agee.2020.106929.

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