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Original Articles

Leaf Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) biochemical profile of grassland plant species related to land-use intensity

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ABSTRACT

There is growing interest in the application of plant functional trait-based approaches for development of sustainable land-use strategies. In this context, one crucial task is to identify and measure plant traits, which respond to land-use intensity (response traits) and simultaneously have an impact on ecosystem functions (effect traits). We hypothesized that species-specific leaf chemical composition, which may function both as response and effect trait, can be derived from Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy tools in combination with multivariate statistical methods We investigated leaf ATR-FTIR spectra of two grasses, *Poa pratensis* L. and *Dactylis glomerata* L., and one forb, *Achillea millefolium* L. collected in grassland plots along a land-use intensity gradient in three regions of Germany. ATR-FTIR spectra appear to function as biochemical fingerprints unique to each species. The spectral response to land-use intensity was not consistent among species and less apparent in the two grasses than in the forb species. Whereas land-use intensification enhanced protein and cellulose content in *A. millefolium*, giving rise to changes in six spectral bands in the frequency range of 1088–1699 cm^{-1}, only cellulose content increased in *D. glomerata*, affecting the bands of 1385–1394 cm^{-1}. *Poa pratensis* spectra exhibited minimal changes under the influence of land-use, only in the spectral bands of 1373–1375 cm^{-1} associated with suberin-like aliphatic compounds. Our findings suggest that some species' leaf chemical composition is responsive to land-use intensity, and thus, may have a predictive value for ecosystem services provided by those species within grassland vegetation (i.e., herbage yield quality).

1. Introduction

Temperate grasslands are some of the least protected ecosystems worldwide (Hoekstra et al., 2005). In Central Europe, they are habitats for a large range of plant species, with high diversity at small spatial scales (Heijcman et al., 2013). For more than five decades, the vast majority of these grasslands have been intensively managed by intrusive practices such as fertilization, pesticide application, overgrazing and mowing (Isselstein et al., 2005). One important consequence of land-use intensification has been the loss of biodiversity (Duraiappah and Naeem, 2000). Plant species differ in their functional traits, resulting in species-specific contributions to overall ecosystem function (Maskell et al., 2009; Jones et al., 2014). Therefore, loss of biodiversity leads to changes in community trait composition (Chapin et al., 2000), with potential consequences for ecosystem functioning (Sala et al., 2000; Foley et al., 2005). Principles of functional trait-based ecology have become increasingly important in development of sustainable management for grassland ecosystems (Garnier and Navas, 2012; Wood et al., 2015; Faucon et al., 2017). The key step in this approach is to identify easily measurable plant functional traits which simultaneously explain plants' responses to land-use and their effects on ecosystem function (Lavorel and Garnier, 2002; Martin and Isaac, 2015). Key plant traits are related to leaf chemical composition, and thus, leaf chemical composition can be considered a functional trait itself (Wood et al., 2015). Many chemical leaf traits are reflected in their leaf economic spectrum, such as leaf nitrogen or carbon to nitrogen ratio (Wright et al., 2004; Díaz et al., 2016). Leaf traits are strongly influenced by site conditions and management (Van Soest, 1994; Klaus et al., 2012; Herz et al., 2017a,b). Leaf chemical composition is the major component of herbage quality, directly contributing to feed resource quality and,

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subsequently, to livestock production (Barnes et al., 2003), so is directly linked to ecosystem services (Jones et al., 2014). Leaf chemical composition of both single species (Peyraud et al., 1997; Baranauskiene et al., 2003; Nguyen and Niemeyer, 2008; Herz et al., 2017a) and communities (Klaus et al., 2012; Breitschwerdt et al., 2015) has been investigated with respect to alteration of soil nutrient availability via fertilization as well as other effects of land-use changes (e.g., changes in species richness, Klaus et al., 2012). However, to extend the concept of using leaf chemical composition as a response and effect trait at the ecosystem level, knowledge of trait variation at the level of individual plants within distinct species in a grassland community is required. To date, leaf chemical composition has been considered a *hard* trait (Lavorel and Garnier, 2002), informative with respect to its functional role but difficult to measure for large sets of species (Hodgson et al., 1999; Weiher et al., 1999).

In this study, we attempt to solve this problem by using Attenuated Total Reflection Fourier Transform Mid-Infrared (ATR-FTIR) spectroscopy, a fast and cost-effective tool requiring minimal sample preparation, to investigate the response of leaf chemical composition to land-use intensity. ATR-FTIR has been widely used for the chemical analysis of biological samples (Movasaghi et al., 2008; Berthomieu and Hienerwadel, 2009). Infrared absorption spectra reveal the characteristic molecular vibrational transitions of specific functional groups of chemical compounds, providing a comprehensive biochemical fingerprint of the analyzed sample (Griffiths, 1978; Baker et al., 2014). In contrast to the more widely used near-infrared (NIR) region, where absorption bands represent the overtones, the mid-infrared is the region of fundamental molecular vibrations (Smith 1979); consequently, the absorbance band are less overlapped and better distinguished in the mid than NIR spectral ranges. In the last decade, ATR-FTIR spectroscopy was successfully applied to the taxonomical discrimination of plant species, demonstrating its reliability to probe plant chemical composition as a species trait (Kim et al., 2004; Rana et al., 2008; Meinen and Rauber, 2015). Combining ATR-FTIR spectral output with multivariate analysis, we tested the hypothesis that plant chemical response to land-use intensity is consistent among species. We focused on two grasses, *Poa pratensis* L. (Kentucky bluegrass) and *Dactylis glomerata* L. (cocksfoot), and one forb, *Achillea millefolium* L. (yarrow) which are common European grassland species that occur under various land-use conditions (Gudzenko, 2013; Huo et al., 2013). *P. pratensis* and *D. glomerata* are forage plants of high dry matter production with economic importance (Rumball, 2005; Huff, 2010; Simili da Silva et al., 2013). *A. millefolium* is a well-known medicinal plant containing essential pharmacologically active compounds (Nemeth and Bernath, 2008). The objective of our study was i) to assess the potential of ATR-FTIR spectra of leaf samples as a species' biochemical fingerprint, and ii) to relate components of the species' ATR-FTIR spectra to land-use intensity.

To this end, we recorded ATR-FTIR spectra of field collected leaf samples of *P. pratensis*, *D. glomerata*, and *A. millefolium* phytometers, planted into grasslands under a gradient of land-use intensity.

2. Materials and methods

2.1. Study area and land-use intensity

The study was carried out in three regions of Germany as part of the DFG-Biodiversity Exploratories Project (www.biodiverity-exploratories.de; Fischer et al., 2010). Each region consists of 50 grassland plots (50 m × 50 m), which span a land-use gradient, ranging from plots with intensive management and fertilization to those with low management intensity and without fertilization. The southernmost region, Schwäbische Alb, is situated in a low mountain range in southwest Germany (09°10′49″–09°35′54″ E/48°20′28″–48°32′02″ N, 460–860 m a.s.l., mean annual temperature 8-8.5 °C, mean annual precipitation 800–930 mm, soils mainly leptosols and cambisols). Hainich-Dün region is located in a hilly area in central Germany (10°10′24″ – 10°46′45″ E/50°56′14″–51°22′43″ N, 285–550 m a.s.l., mean annual temperature 6.5–8 °C, mean annual precipitation 630–800 mm, soils mainly cambisols and stagnosols). The northernmost region, Schorfheide-Chorin, is situated in the lowlands of northeast Germany (13°23′27″–14°08′53″E/52°47′25″–53°13′26″ N, 3–140 m a.s.l., mean annual temperature 6–7 °C, mean annual precipitation 520–580 mm, soils mainly drained histosols, lavisols or gleysols). For further details see Fischer et al. (2010).

The grasslands are managed by mowing, grazing or a combination of both, and are either unfertilized or fertilized at different intensities. Information on land-use is constantly collected from landowners and managers using standardized questionnaires (Blüthgen et al., 2012). This information has been summarized in a Land-Use-Intensity index (LUI, Blüthgen et al., 2012). The LUI index consists of the sum of intensity-standardized measures of three components; fertilization, mowing, and grazing. Fertilization intensity is the amount of nitrogen added per hectare during the year (kg nitrogen ha^{-1} $year^{-1}$, as chemical fertilizer, manure or slurry). Mowing intensity is the number of cuttings per year. Grazing intensity represents the density of cattle, sheep, or horses per hectare over the year (livestock unit days of grazing ha^{-1} $year^{-1}$). The three components were individually standardized by dividing the value obtained for each plot by the mean values obtained from the respective region (i.e. the mean of all 50 experimental plots in the Schwäbische Alb, Hainich-Dün or Schorfheide-Chorin). For further details, see Blüthgen et al. (2012). The mean LUI value was calculated for the interval 2006–2014.

2.2. Plant material and sampling

The selected species are among the most frequent and abundant plants in the grassland plots of the German Biodiversity Exploratories Priority Programme. To avoid differences in growth stage among plants that may alter leaf structural carbohydrate and lignin content (Barnes et al., 2003; Kellems and Church, 2003), *Achillea millefolium* L. (Asteraceae), *Dactylis glomerata* L., and *Poa pratensis* L. (Poaceae) were introduced as phytometers planted into grassland plots in the three regions. The plants were raised from seeds previously collected in those plots or purchased (for *P. pratense*, by Rieger-Hofmann GmbH, Blaufelden, Germany), in the greenhouse of the Botanical Garden Halle (Germany) from December 2013 to April 2014. Phytometers were planted in the field in May to June 2014 (for details see Herz et al., 2017a,b). Five individuals of each phytometer species were planted in 54 plots. We harvested one individual of 22 plots, spanning a full range of LUI values (0.5–2.6) across the three regions (Table S1). Thus, all species were exposed to the same site conditions and land use management as the resident species in the different plots. Plants were harvested in August to September 2014. The leaf material was immediately frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.3. Sample preparation and ATR-FTIR spectra acquisition

The leaf material was freeze-dried (P4K-S, Dieter Piatkowski Forschungsgeräte Vertrieb, München, Germany), and subsequently milled to a fine powder for 105 s at 25 cycles per second speed using a ball mill (type MM2, Retsch, Haan, Germany). To prevent any heating during milling, the procedure was conducted using liquid nitrogen to permanently cool down the tube recipients and the balls.

ATR-FTIR spectra for both background and leaf powder measurements were recorded at a resolution of 4 cm^{-1} and 32 scans in the range of 600–4000 cm^{-1}. The recording was done with the FTIR spectrometer Equinox 55 (Bruker Optics, Ettlingen, Germany) with a deuterium triglycine sulfate detector and an attached ATR unit (DuraSamplIR, SensIR Europe, Warrington, UK). The samples were pressed against the zinc selenide (ZnSe) crystal of the ATR device until a torque knob ensured that the pressure applied was the same for all