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# Visualizing land-use and management complexity within biogeochemical cycles of an agricultural landscape

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Abstract. Crop fields are cultivated across continuities of soil, topography, and local climate that drive biological processes and nutrient cycling at the landscape scale; yet land management and agricultural research are often performed at the field scale, potentially neglecting the context of the surrounding landscape. Adding to this complexity is the overlap of ecosystems and their biogeochemical legacies, as a patchwork of crops fields, natural grasslands, and forests develops across the landscape. Furthermore, as new technologies and policies are introduced, management practices change, including fertilization strategies, which further alter biological productivity and nutrient cycling. All of these environmental, biological, and historical legacies are potentially recorded in the isotopic signal of plant, soil, and sediment organic matter across the landscape. We mapped over 1500 plant, soil, and sediment isotopic values and generated an isotopic landscape (isoscape) over a 40-km<sup>2</sup> agricultural site in NE Germany. We observed distinct patterns in the isotopic composition of organic matter sampled from the landscape that clearly reflect the landscape complexity.  $C_3$  crop intrinsic water-use efficiency reflected a precipitation gradient, while native forest and grassland plant species did not, suggesting that native plants are more adapted to predominant climatic conditions.  $\delta^{13}C_{soil}$  patterns reflected both the long-term input of plant organic matter, which was affected by the local climate conditions, and the repeated cultivation of corn. Soil organic matter <sup>15</sup>N isotopic values also revealed spatial differences in fertilization regimes. Forest fragments, in which the nitrogen cycle was relatively open, were more water-use efficient. Sediments from small water bodies received substantial inputs from surrounding field vegetation but were also affected by seasonal drying. These isotopic maps can be used to visualize large spatial heterogeneity and complexity, and they are a powerful means to interpret past and current trends in agricultural landscapes.

**Key words:** agricultural landscape; isoscape; land management; land-use change and impacts; spatial visualization; stable isotopes.

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### INTRODUCTION

Societal demands for food, energy, shelter, and commerce have resulted in the modification of landscapes globally (Tilman et al. 2002). The ecosystem services these landscapes yield has become a point of focus as climate change and land-use disrupt essential landscape functions (DeFries et al. 2004, Foley et al. 2005). Land-use is the modality in which the land cover is changed for human purposes including management practices (Foley et al. 2005, Verburg et al. 2011). Land-use changes not only the physical appearance of the land cover but also intrinsic properties, such as plant communities, soil development, and water and nutrient cycles. Furthermore, the landscape is often broken into management units creating abrupt transitions between land-use types and landscape elements. Thus, the intensity and duration of land-use and management across a landscape is not homogenous despite the underlying connectivity in terms of topography, ground water, and soil type.

Agricultural landscapes are complex mosaics of overlapping types of land-use, ecosystems, and soils and can be characterized by high levels of heterogeneity (Roschewitz et al. 2005). Past estimates of agricultural and pastureland range from 30% to 40% of the earth's ice-free land (Olesen and Bindi 2002, Foley et al. 2005), and agricultural land is under pressure to increase production and mitigate effects from climate change (West et al. 2010). Strategies to fulfill this goal include the introduction of improved crop varietals optimized for productivity, fertilizer application, irrigation, crop rotation, and land conversion, all of which impact or leave a legacy on local biogeochemical cycles (Olesen and Bindi 2002, Tilman et al. 2002). Thus, to assess current land management and policies, we need tools for observing and quantifying the potential biogeochemical impacts within and across different landscape elements, such as forests, grasslands, and water bodies, challenging researchers to develop measurements and proxies that reflect both biological and environmental patterns at broad spatial scales.

Generating isoscapes (i.e., isotopic landscapes) from natural abundance stable isotopes is one approach to understanding biogeochemical responses both spatially and quantitatively (Bowen

2010). Physical and biochemical transformations that are influenced by the environment and organism metabolism are imprinted on the isotopic value (ratio of heavy to light isotope referenced to a) of organic matter (Lichtfouse et al. 1995, Brüggemann et al. 2011, Werner et al. 2012). Plants within the agricultural landscape, including crop and plant species native to forests and grasslands, are therefore in situ biological recorders of changes in precipitation regime, temperature, light, and CO<sub>2</sub> concentrations (Tubiello et al. 2007, Hatfield et al. 2011), as well as landmanagement practices (Bateman et al. 2005, Choi et al. 2006). Soil organic matter is an amalgam of recent plant litter, microbial products, and management inputs that are stored and released over time providing information about current and past conditions.

When the isotopic composition of these different sources are used in combination with wellconstrained isotope fractionation models (e.g., discrimination associated with photosynthesis), then the isotopic signal of a relatively few representative samples can be extrapolated over space allowing for the interpretation of different physical and biological processes. Isoscapes have been created at local scales to track the spatialtemporal impact of exotic N2-fixing invaders (Rascher et al. 2012, Bai et al. 2013), at regional and continental scales to identify source water dynamics of rivers (Kendall and Coplen 2001, Brooks et al. 2012), and at global scales to reveal plant responses to climate (Amundson et al. 2003, West et al. 2008).

While the isotope values (<sup>13</sup>C and <sup>15</sup>N) of organic matter alone can yield information about changes across the landscape, they can also be used to derive proxies of plant stress and nutrient cycles. Intrinsic water-use efficiency (WUE<sub>i</sub>) is one such proxy that describes the ratio of carbon assimilated to water transpired, which directly links water stress during photosynthesis to the  $\delta^{13}$ C signals of plant leaves (Saurer and Siegwolf 2007, Seibt et al. 2008). Plant <sup>15</sup>N normalized by soil <sup>15</sup>N ( $\Delta\delta^{15}$ N) is another integrative index that has been positively correlated with increased N availability through mineralization (Kahmen et al. 2008), N transformations through nitrification (Takebayashi et al. 2010) and N losses through denitrification (Houlton and Bai 2009), and leaching (Cheng et al. 2010, Fang et al. 2010).



Fig. 1. Land-use map from 2013 (a) showing the three dominant land-use types: grasslands, forests, and arable fields including the crops cultivated in 2013 and villages (ZALF Research Station, Dedelow, and Department of Landscape Information Systems, ZALF, Muencheberg). Location of the study site (red rectangle) in the federal state of Brandenburg within Germany (b). Note that the study site map has been rotated 40° for horizontal display, and a NW to SE transect has been added above the map to further orient the reader.

Thus, isotopic values and proxies that capture these biological and physical processes are proven tools to interpret land-management activities, such as the application of chemical and organic fertilizers (Yoneyama et al. 1990, Choi et al. 2003) and tillage leading to erosion (Dungait et al. 2013, Beniston et al. 2015).

Isoscapes have the potential to help understand complex land-use and management patterns of agricultural landscapes, but little is known about the heterogeneity of organic matter across fields and landscape structures. Based on a high-spatial resolution isotopic ( $\delta^{13}$ C and  $\delta^{15}$ N) data set, we created plant and soil isoscapes of a moraine landscape of NE Germany. The landscape is primarily used for agriculture (Fig. 1) and consists of numerous landscape structures (e.g., arable fields, grasslands, forests, and villages), including kettle holes. Kettle holes are small water bodies (<1 ha in size) present across different land-use types that function as internal drainage systems (Kalettka et al. 2001). Kettle holes are hypothesized to be important hot-spots of biogeochemistry (Lischeid and Kalettka 2012), thus providing an opportunity to test for terrestrial-aquatic linkages at the landscape scale. Using isoscapes we expect to: 1) observe plant and soil patterns across different landscape structures in response to climate or past land-use thereby revealing landscape connectivity; 2) detect different land-management practices and land-use effects on carbon and nitrogen biogeochemical cycles; and 3) explore whether kettle hole sediments and surrounding plants and soil exhibit tight aquatic-terrestrial linkages.

# MATERIAL AND METHODS

### Study site

The study site is located within the Quillow catchment (168 km<sup>2</sup>) and is part of the complex hummocky young ground and terminal moraine landscape typical for NE Germany. The catchment contains typical elements of post-glacial landscapes including eroded hilltops,

slopes, plains, and depressions (Sommer et al. 2008). Arable land use began in the area after massive deforestation in the 12th century. The study site is one of the most fertile areas in NE Brandenburg (D. Barkusky, *personal communication*). The climate is subhumid with a mean annual temperature of 8.6°C and mean annual precipitation varies between 350 and 750 mm/yr.

The proportions of land-use types across the study site were 74.4% arable, grasslands 10.4%, and forest 5.9%. Towns and industrial areas (4.5%) and larger stagnant water bodies of >1 ha (moors, lakes, reed dominated; 3.4%) were also dispersed over the area (Fig. 1). Kettle holes made up approximately 1.4% of the area and were present in all land-use types. In general, the NW and center contained more water bodies, including lakes and moors. Arable soils consisted of albeluvisols with minor arenosols, luvisols, and planosols. Grasslands consisted of stagnant planosols and albeluvisols as well as histosols of peat origin. Forests consisted of planosols and albeluvisols including luvisols and arenosols in minor proportions. Forest areas within the site varied by size. The largest forest (approximately 261 ha) located within our site was in the SE, followed by a 158-ha forest in the NW. We refer to these two forests as "intact" due to their large size. These two larger forests only partly extended into our site, while two others (71 and 34 ha) were located entirely within the sampling area. Based on local weather station data spanning from 1981 to 2010, a 45-mm gradient in precipitation exists from 590 mm in the NW to 545 mm in the SE (see Appendix S1: Fig. S1). The landscape is moderately rolling with elevations ranging from 40 to 112 m a.s.l. and a general downward slope of 0.15° to the SE.

#### Sampling design

We divided the isoscape study area  $(3.25 \times 11.75 \text{ km}; 38.2 \text{ km}^2)$  into 611 subplots each 0.25 × 0.25 km<sup>2</sup>. This yielded an average of ~4.7 sampling points within each polygon (i.e., borders of fields and different land-use types depicted in Fig. 1). Because few isoscapes have the same spatial scale as our study, we compiled sample densities from previous studies that range in spatial extent from <1 km<sup>2</sup> to the globe for comparison (Appendix S1: Fig. S2).

Our sampling density was above average given the area of our investigation, thus assuring at least a similar representation to previous isoscape research.

Samples were taken from the center of each accessible subplot (Appendix S1: Fig. S3). Not all grid points were sampled. Towns, industrial grounds, and streets were omitted, as were protected grasslands and wetlands >1 ha. When sampling points were positioned along a boundary of two different land-use types, then one sample of each land-use type was taken approximately 20 m from the boundary. If the center of a polygon could not be reached, the sample was taken as close as possible (c. 50 m) to the original sampling coordinate. Arable fields were relatively large and the range of samples taken from one field varied from 1 to 22 depending on size and shape.

During the 2013 growing period (May/June), we collected 595 soil samples and 589 plant samples (499 crop, 57 grassland, and 34 forest). In agricultural fields, we sampled crops, dandelion (Taraxacum sect. Ruderalia) on grassland sites, and beech (Fagus sylvatica) in forests. Beech trees were usually young and less than 2 m in height. Crop species included winter wheat (Triticum L.), winter barley (Hordeum vulgare), winter rye (Secale cereale), oats (Avena sativa), rapeseed (Brassica napus), sugar beet (Beta vulgaris), and corn (*Zea mays*). Our plant samples were composites of leaves from three different plants near the sampling point. For soils, we assumed that the plow layer (30 cm) is well mixed in arable fields and provide a representative sample of land-use and management effects on the soil. We took 200–300 g soil sample from 2 to 15 cm depth for all land-use types after removing the top 2 cm, which was rich in fresh plant residues from the previous growing period.

From mid-July to the end of August, we sampled sediment (51) and plants (34) from 51 kettle holes out of the approximately 290 present. We sampled *Phalaris arundinacea* to represent a kettle hole plant sample because it was present at almost all sites. We based our kettle hole selection on (1) size class, ensuring that we covered the distribution of different size classes, (2) land-use type, and (3) spatial distribution (we ensured to cover the study area; Appendix S1: Fig. S3). We mixed the top 2 cm from three sediment cores for each kettle hole sediment sample.

### Sample preparation

All samples were stored at 4°C in dark conditions prior to preparation. Plant, soil, and sediment samples were oven-dried at 65°C for 48 h. We ground plant and sediment samples to a powder. Soil samples were passed through a 2-mm sieve and visible plant residues were removed. To identify samples for acid fumigation, we determined carbonate concentrations (in %  $CO_3$ -C) in soil and sediment samples using a RC612 multiphase carbon and nitrogen analyzer (LECO Corporation, Michigan, USA).

# Stable isotope analysis

Prior to  $\delta^{13}C$  stable isotope analysis of soil samples, inorganic carbon was removed from samples containing >0.05% CO<sub>3</sub>-C by acid fumigation after Harris et al. (2001). Soil, plant, and sediment samples were weighed into tin capsules. All isotope measurements were analyzed on a Thermo-Scientific, Delta V advantage isotope ratio mass spectrometer. The isotopic values are expressed in delta notation (in ‰ units), relative to VPDB (Vienna Pee Dee Belemnite) for carbon and N<sub>2</sub> in air for nitrogen. Analysis of internal laboratory standards (apple leaves, ulva, high organic carbon content sediment, low organic carbon content soil) ensured that the estimates of the isotopic values were accurate to within <0.1‰ for  $\delta^{13}$ C and <0.5‰ for  $\delta^{15}$ N.

### Plant physiological-related calculations

We calculated the photosynthetic carbon isotope discrimination  $\Delta^{13}$ C between the ambient atmospheric  $\delta^{13}C_a$  (-8‰) and the measured  $\delta^{13}C_{\text{plant}}$  of C<sub>3</sub> plants (Farquhar and Richards 1984):

$$\Delta^{13}C = \frac{\delta^{13}C_a - \delta^{13}C_{plant}}{1 + \delta^{13}C_{plant}/1000} \tag{1}$$

The intrinsic water-use efficiency (WUE<sub>*i*</sub>) in  $\mu$ mol/C mol is calculated on the basis of the measured plant  $\delta^{13}$ C following the equation of Saurer and Siegwolf (2007):

$$WUE_i = \frac{c_a}{1.6} \cdot \frac{b - \delta^{13}C_a + \delta^{13}C_{plant}}{b - a}$$
(2)

Here,  $c_a$  is the ambient atmospheric concentration of CO<sub>2</sub> of 380 µmol/mol, *a* is the C isotope fractionation during stomata CO<sub>2</sub> diffusion (4.4‰), *b* represents the C isotope fractionation during the CO<sub>2</sub> fixation during RuBisCO (27‰), and the value of 1.6 is the ratio of the diffusivities of CO<sub>2</sub> and water in air.

In terms of connecting soil and plant  $\delta^{15}N$ , we calculated the difference between both compartments ( $\Delta\delta^{15}N$ , Amundson et al. 2003):

$$\Delta \delta^{15} N = \delta^{15} N_{plant} - \delta^{15} N_{soil} \tag{3}$$

The resulting value is also a proxy for the status of nitrogen cycling. Due to fractionation involved during the formation of nitrogen sources belowground, especially those associated with denitrification (Kahmen et al. 2008), leaves tend to be depleted in <sup>15</sup>N compared to bulk soil values (Robinson 2001). Thus, when  $\Delta\delta^{15}$ N values are near 0‰, resulting from an enriched plant isotopic value, then the N cycle is considered to be open, indicating the possible occurrence of nitrogen sources depleted in <sup>15</sup>N leaching or that different N sources are available (Zech et al. 2011).

#### Statistical analysis

To test statistical significances between plant and soil  $\delta^{13}C$  and  $\delta^{15}N$  across our three landuse types and for kettle hole sediments, we performed ANOVA, post hoc significance test (Tukey's HSD) and linear regression using R (version 3.1.1, R Foundation for Statistical Computing, Vienna, Austria, http://www.Rproject.org/). Interpolations were made using ordinary kriging within the Geostatistical Analyst function of ArcGIS geographic information system software (version 10.1, Environmental Systems Research Institute 2012, Redlands, CA). We omitted wetlands of >1 ha (lakes, reeddominated areas, moors) and urban areas from interpolation. We created our WUE, plant isoscapes by interpolating across C<sub>3</sub> crops in arable land-use types, beech in forests, and dandelion in grasslands. Land-use boundaries were based on a land-use map from 2013. Subsequently, we compiled all land-use interpolations into an overall WUE<sub>i</sub> isoscape. We used a single scale to achieve a better visualization of patterns across the three land-use types, which still allows for discerning patterns within each single land-use type. We followed the same procedure

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Land use	δ <sup>13</sup> C (‰)	δ <sup>15</sup> N (‰)	Δδ <sup>15</sup> N (‰)
Soils			
Arable $(n = 497)$	$-26.5\pm0.0$	$6.1 \pm 0.1$	$-2.7 \pm 0.1$
Forest $(n = 38)$	$-27.0\pm0.1$	$0.8 \pm 0.3$	$-5.4 \pm 0.4$
Grassland $(n = 60)$	$-27.5\pm0.1$	$4.7 \pm 0.1$	$-2.7 \pm 0.3$
Sediments			
Arable $(n = 36)$	$-28.7\pm0.2$	$3.7 \pm 0.2$	
Forest $(n = 6)$	$-28.5 \pm 0.3$	$-0.3 \pm 0.3$	
Grassland $(n = 9)$	$-28.4\pm0.3$	$2.8 \pm 0.3$	

Table 1. Soil and sediment  $\delta^{13}C,~\delta^{15}N$  (‰), and  $\Delta\delta^{15}N$  (‰) mean ± SE.

for the creation of our  $\Delta \delta^{15}$ N,  $\delta^{15}$ N plant, and soil isoscapes. However, for the  $\delta^{13}C_{soil}$  isoscape, we did not divide the landscape by land use since the difference between mean  $\delta^{13}C_{soil}$  for each land-use type was not greater than 1‰ (Table 1).

### Mixing model

We assumed three primary  $\delta^{13}C$  and  $\delta^{15}N$ organic matter sources to the kettle hole sediments: surrounding soil, kettle hole plants, and surrounding vegetation in the fields (e.g., crops, grasses). We used the SIAR mixing model package that is implemented in R (Parnell et al. 2008). The model allows for the estimation of a fractionation factor associated with the transformation of plant or soil organic matter before it reaches the sediment. We chose conservative fractionation values of 0.7‰ for  $\delta^{13}C$ (Brüggemann et al. 2011) as well as for  $\delta^{15}N$ (Yoneyama 1996). The software reports credibility intervals (95%, 75% and 25%) for the partitioning results.

# Results

# Plant $\delta^{I3}C$ isoscape patterns

We grouped plants by land-use type for interpolation of isotopic values and associated proxies across the site. For the arable land-use type, we grouped our C<sub>3</sub> crops based on estimates of photosynthetic discrimination ( $\Delta^{13}$ C). Photosynthetic discrimination was on average -21.0 ± 0.1‰ and the total range did not exceed ±1.5‰. Plant isotopic values (Table 2) exhibited patterns across the research site that reflected climate and differences in vegetation. Interpolated  $\delta^{13}$ C<sub>plant</sub> of arable

Table 2. Plant leaf  $\delta^{13}$ C,  $\delta^{15}$ N (‰), and  $\Delta\delta^{15}$ N (‰) mean ± SE of different species.

Plant	Leaf δ <sup>13</sup> C (‰)	Leaf δ <sup>15</sup> N (‰)	Δδ <sup>15</sup> N (‰)
$C_3 \text{ crops } (n = 386)$	$-29.0 \pm 0.1$	$3.4 \pm 0.1$	$-2.6 \pm 0.1$
Barley $(n = 56)$	$-29.1\pm0.1$	$2.1 \pm 0.4$	$-3.8 \pm 0.3$
Oats $(n = 7)$	$-29.9\pm0.2$	$4.8 \pm 0.7$	$-1.1 \pm 0.9$
Rapeseed $(n = 110)$	$-29.9\pm0.1$	$4.7 \pm 0.2$	$-1.4 \pm 0.3$
Rye ( <i>n</i> = 12)	$-29.2 \pm 0.2$	$2.2 \pm 0.3$	$-3.1 \pm 0.3$
Sugar beet $(n = 22)$	$-28.9\pm0.2$	$3.7 \pm 0.6$	$-2.9 \pm 0.6$
Wheat $(n = 179)$	$-28.4\pm0.1$	$2.9 \pm 0.1$	$-3.1 \pm 0.2$
$C_4$ crops			
Corn (n = 110)	$-13.2\pm0.0$	$3.5 \pm 0.3$	$-2.7 \pm 0.3$
Forest plants			
Beech $(n = 34)$	$-32.4 \pm 0.2$	$-4.5 \pm 0.4$	$-5.4 \pm 0.4$
Grassland plants			
Dandelion $(n = 57)$	$-30.3\pm0.1$	$2.0 \pm 0.3$	$-2.7 \pm 0.3$
Kettle hole plants			
Phalaris arundinacea	$-27.3\pm0.1$	$5.0\pm0.4$	
(n = 34)			

fields (C<sub>3</sub>) spanned from -31.6 to -26.7‰ (Appendix S1: Fig. S5). For C<sub>3</sub> crop leaf organic matter in general, we found a pattern in  $\delta^{13}$ C depletion from the SE to NW of our site, except for a small area in the NW (Appendix S1: Fig. S4A) where the plants were slightly enriched in <sup>13</sup>C (~1‰) compared to the surrounding fields. A clear trend was not visible for cornfields across the site, and small local variations occurred within each field ranging from -13.7 to -12.7‰ (Appendix S1: Fig. S4C). In the forest land-use type, we found that beech trees in the NW were more enriched relative to forests near the center of the site (Appendix S1: Fig. S4B). Interpolated  $\delta^{13}C_{\text{beech}}$  had a range from -34.7 to -28.3‰. In the grassland land-use type, we found very little variation in the interpolated  $\delta^{13}C$  among the sampled dandelion (-31.3 to -29.3‰) (Appendix S1: Fig. S4B).

### Intrinsic water-use efficiency (WUE<sub>i</sub>)

The patterns in  $WUE_i$  across the arable landuse type show an overall decrease from SE to NW. In addition, interpolated  $WUE_i$  values exhibited a wide range in values of 28–80 µmol/ mol compared with plants from the other landuse types (Fig. 2). Within the forest land-use type, beech was more water-use efficient in the NW compared with the center of the site. Beech



Fig. 2. Isoscapes (a) of intrinsic water-use efficiency (WUE<sub>i</sub>,  $\mu$ mol/mol) for plants sampled from the three land-use types using ordinary kriging. Interpolation was performed first for each land-use type independent of other types. The three isoscapes were then compiled into one overall isoscape using a common scale. Grasslands are highlighted with red borders and forests with black borders. The area interpolated to generate each land-use type isoscape is shown in (b) and includes the same scale. Areas not interpolated include fields with corn, wetlands >1 ha, and urban areas.

exhibited the smallest WUE<sub>i</sub> of all sampled plants with a range of 1–61  $\mu$ mol/mol. Within the grassland land-use type, dandelion was more water-use efficient in the NW compared with the SE and had a range of 31–53  $\mu$ mol/mol.

### Soil $\delta^{13}C$

The overall variation in  $\delta^{13}C_{soil}$  ranged from –28.3 to –24.7‰ with the most enriched values found in the SE of our site. With such a small range in values (Table 1), we interpolated  $\delta^{13}C$  over the whole sampling area without separating areas into different landuse types. Similar to patterns of WUE<sub>i</sub>, there was a small decrease in  $\delta^{13}C_{soil}$  from SE to NW (Fig. 3).

# $\Delta \delta^{15}$ N pattern

We found strong variations in plant and soil  $\delta^{15}$ N among the three land-use types (Appendix

S1: Table S1), consequently we interpolated <sup>15</sup>N within each type. In general,  $\Delta \delta^{15}$ N patterns (Fig. 4) were similar to the foliar  $\delta^{15}N$  pattern (Appendix S1: Fig. S5), indicating the disequilibrium between plant and soil. There was a wide range of  $\Delta \delta^{15} N$  (-5.8 to +0.6‰) within arable fields that was clearly related to crop species. In particular, we found oat and rapeseed to have the least negative values, that is, these were the plant species most enriched in <sup>15</sup>N. Forest  $\Delta \delta^{15}$ N values were the most negative (-7.8 to -3.3‰) and were strongly positively correlated with WUE; of beech (Appendix S1: Fig. S6). The two fragmented forests stands were the most water efficient and exhibited the most open nitrogen cycle between plant and soil (i.e., the  $\Delta \delta^{15}N$  was closest to zero). In the grassland land-use type, the range of dandelion  $\Delta \delta^{15}$ N was -4.5 to -1.1‰, but values were also variable across the site, especially in the NW (Fig. 4).

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Fig. 3.  $\delta^{13}C$  (‰) soil isoscape. Interpolation was performed over the whole-sampling area without separating land-use types. Grasslands are highlighted with red borders and forests with bold-black borders.



Fig. 4. Isoscapes (a) of  $\Delta \delta^{15}$ N (‰) for plants and soils sampled from the three land-use types using ordinary kriging. Interpolation was performed first for each land-use type independent of other types. The three isoscapes were then compiled into one overall isoscape using a common scale. Grasslands are highlighted with red borders and forests with bold-black borders. The area interpolated to generate each land-use type isoscape is shown in (b) and includes the same scale. Areas not interpolated include wetlands >1 ha and urban areas.



Fig. 5. Isoscapes (a) of  $\delta^{15}$ N (‰) for soils sampled from the three land-use types using ordinary kriging. Interpolation was performed first for each land-use type independent of other types. The three isoscapes were then compiled into one overall isoscape using a common scale. Grasslands are highlighted with red borders and forests with bold-black borders. The area interpolated to generate each land-use type isoscape is shown in (b) and includes the same scale. Areas not interpolated include wetlands >1 ha and urban areas. Highlighted areas show land-management effects: grasslands growing on histosols (blue borders) or nonhistosols (light red borders), conversion of arable fields (green borders), application of cattle slurry (violet borders), application of pig slurry, plus organic-rich soils (cyan borders).

# Soil $\delta^{15}N$

The isoscape of  $\delta^{15}N_{soil}$  was interpolated for each land-use type. Interpolated arable field  $\delta^{15}N_{soil}$  values had a range of 3.0-8.5‰ and fields near villages were typically enriched. In the SE, around a wide area of the village Falkenhagen (Fh), we found soils with the most enriched  $\delta^{15}$ N isotopic values (Fig. 5). The area close to the village Kraatz (Kr) in the NW was also enriched in <sup>15</sup>N as well as a small area south of the village Rittgarten (Ri). Soils near the center of the study area and the NW were less enriched in <sup>15</sup>N. Forest soil was the least enriched in <sup>15</sup>N (0 to +3.0%) of all land-use types. The northern most intact forest was most enriched of the four stands. Grassland land-use type soils varied only slightly in their  $\delta^{15}N$ values (4.1-5.2‰), although we observed small

differences among the different locations. Grasslands in the west were enriched in <sup>15</sup>N relative to those in the east.

### Terrestrial-aquatic linkages

We could not detect a difference in sediment  $\delta^{13}$ C among the three land-use types; however, we did find differences based on  $\delta^{15}N_{sediment}$  values (Appendix S1: Table S1). Forest kettle hole sediment  $\delta^{15}N$  isotopic values were significantly different than grassland (P < 0.05) and arable (P < 0.01) kettle hole sediments. We could not detect a significant difference between sediments of kettle holes from grassland and arable land-use types. Given the land-use type differences between sediments and the surrounding vegetation isotopic composition, we found it reasonable to analyze

the sediment-plant-soil relationship within the three land-use types.

We tested for linkages between terrestrial plant and soil organic matter sources to the kettle hole sediments. We assumed at steady-state the kettle hole sediments would reflect these dominant sources. The soil and plant sources near the kettle hole were isotopically similar, and often the sediments were relatively depleted in the heavy isotope, while plant organic matter from the fields, grasslands, and forests were the most depleted (Fig. 6). The sediment  $\delta^{13}$ C and  $\delta^{15}$ N mixing-model results (Appendix S1: Fig. S7a-c) largely reflected these different plant sources, although the mixing patterns are primarily driven by variation in  $\delta^{15}$ N. Within the arable land-use type, organic matter from the surrounding  $C_3$ crops contributed between 90% and 95% to the sediment organic matter isotopic value. In contrast, the sediment isotopic composition from



Fig. 6. Biplot of plant, soil, and sediment isotopic composition used in kettle hole mixing-model analysis. Different land-use types are identified by color: blue (arable), red (grassland), and green (forest). Enlarged symbols are the median for each group. Kettle hole (KH) plants are represented by Phalaris arundinacea in arable fields and grasslands. Isoscape plants are represented by sampled C3 crops in arable fields, dandelion in grasslands, and beech in forests. Soils represent mean values of four soil isoscape samples nearest to a kettle holes. For arable fields, we constrained soil values to isoscape sampling points within the same field as the kettle hole.

the grassland land-use type received a large contribution from the surrounding vegetation (50-90%; 95% credibility interval) and only a smaller contribution from vegetation growing directly in the kettle hole vicinity. Sediment  $\delta^{15}N$  from one kettle hole was significantly (> $2\sigma$ ) enriched compared with the others and was removed from the source partitioning analysis. For the forest landuse type, there were only two sources, beech leaves and soil, that could contribute to the sediment isotopic value. The soil was estimated to contribute less (5–60%, 95% credibility interval) than the contribution from beech leaves (40–95%; 95% credibility interval). In general among all the kettle holes, we found the most enriched  $\delta^{15}N$ sediments to originate from kettle holes that tend to dry out over the year.

### Discussion

We observed distinct patterns in the isotopic composition of organic matter sampled from the landscape that clearly reflect the complexity of land-use, land management, and local environmental conditions. Water-use efficiency and nutrient dynamics between crop and native plants were different, leaving a lasting imprint on the soil organic matter. From the subsequent soil organic matter patterns, we were able to observe land management effects that extended beyond a single field or a single growing season. We found the kettle hole sediment isotopic values exhibited a high range of variability across the site that largely reflected the surrounding land-use. In the following, we discuss these two primary drivers of the heterogeneity across the isoscapes: the abiotic environmental conditions and land management, and their overall impact on plant, soil, and sediment of different land-use types.

### Plant and soil response to the environment

Crop  $C_3$  plants from the eastern area of our site were more affected by lower water availability than native plants. The range of WUE<sub>i</sub> for native plants (beech, dandelion) was smaller than the  $C_3$  crops indicating that these plants were perhaps under less water stress than their cultivated counterparts (Grossiord et al. 2014). Plants in the arable fields may face water stress from management practices resulting in less organic matter in the upper soil horizons thereby reducing soil moisture holding capacity (Hudson 1994, Emerson 1995) or the roots from the crop plants may not reach deeper soil water sources available to native species. The C<sub>3</sub> crop plants WUE; patterns also followed a small 45 mm precipitation gradient across our site, while native species, including those surrounding kettle holes, did not. Besides water sources, genetic selection of crop traits to endure water stress may also explain the different response between native and crop plants. Indeed, native plants have been recognized to exhibit fitness homeostasis to fluctuating environmental conditions with respect to invasive species (Davidson et al. 2012), and while crop species are not invasive species per se, they have been developed for specific traits and performance (Garnier and Navas 2011, Varshney et al. 2011) that may differ from native species. Thus, we hypothesize that native plants are more adapted to predominant climatic conditions than crops at the landscape scale.

The soil organic matter isoscapes integrate plant and management inputs over time and are therefore a conservative proxy of their impact, which could lead to a diffuse signal within a landscape. However, the soil organic matter isotopic patterns provided a clear indication of the environmental gradient and land management at our site. For example, the  $\delta^{13}C_{soil}$  values of arable land reflected the precipitation gradient, most likely resulting from organic matter deposited from plants that have responded physiologically to the local environmental conditions. There was a slight correlation of  $\delta^{13}C_{soil}$  with precipitation  $(r^2 = 0.27)$ , which we infer as the long-term influence of plant organic matter on the  $\delta^{13}C_{soil}$  signal. Land management, in terms of corn cultivation, is also evident. Based on land-use maps between 1999 and 2012, we found a strong correlation between  $\delta^{13}C_{soil}$  and corn rotation ( $r^2 = 0.97$ , P < 0.01, Appendix S1: Fig. S8). The effect of corn on  $\delta^{13}C_{soil}$  values is relatively small after only a few rotations (~1‰, Fig. 3), but after four rotations, the impact on  $\delta^{13}C_{soil}$  was larger than 1‰. However, we are cautious with our interpretation since only five fields were planted more than four times with corn. Soil type and the topographic position, generally known to influence  $\delta^{13}C$ and  $\delta^{15}N$  of soils (Fox and Papanicolaou 2007, Alewell et al. 2009), were not highly correlated with  $\delta^{13}C_{soil}$  and  $\delta^{15}N_{soil}$ , another indication of the strong role of land-use and management. The approximate 0.15° slope across the whole site may not induce erosion dynamics that are strong enough to alter the isotopic composition of soil organic matter at our spatial resolution. Overall, we hypothesize that  $\delta^{13}C_{soil}$  patterns reflect both the long-term accumulation of  $\delta^{13}C_{plant}$  affected by the climatic conditions and the imprint of the distinct  $C_4$  isotope value of corn.

### Land-use and land-management effects

There was a strong interaction between landuse and land management. We categorized land-use by the predominant vegetation cover type (forest, arable, and grasslands), and within each, there was a different land-use legacy that formed the backdrop for recent management effects. We observed strong spatial variations in  $\Delta \delta^{15}$ N at the field scale that could not be explained by plant-specific differences in the discrimination against <sup>15</sup>N during N uptake; thus, we found the <sup>15</sup>N of organic matter to respond most strongly to management effects.

In the 1970s within arable fields in Brandenburg, farmers began to intensively apply organic manure, which generally results in enriched  $\delta^{15}N_{soil}$  (Yoneyama et al. 1990, Choi et al. 2003, Bateman et al. 2005), and thus the level of fertilization intensity and type of fertilization was visible in our isoscapes. While we were not able to measure the different varieties of slurry used in the area, we find it reasonable to assume a relatively enriched  $\delta^{15}N$  isotopic signal given the many biological, chemical, and physical processes involved to generate slurry. For example, within the village Dedelow (De) located near the SE boundary of the study area is a large dairy farm (>2000 livestock). Slurry originating from the dairy is transported underground via pipes to fields near the village Falkenhagen (Fh) and distributed on the fields with tractors. We also found soils near the village Kraatz (Kr) enriched in <sup>15</sup>N (Fig. 5) that are known to receive cattle slurry, again providing evidence of slurry application prior to German reunification. Soils from the field south of the village Rittgarten (Ri) are also enriched in <sup>15</sup>N; in this instance, the soil is reflecting the practice of pig slurry application

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occurring after the German Democratic Republic (GDR) period.

In addition to slurry, other fertilization practices at the site were captured by the isoscapes. For example, a field south of Rittgarten (Ri) is fertilized with a peat soil originating from a mushroom farm in addition to pig slurry application (Fig. 5). Fields that were not known to be fertilized by slurry tended to be less enriched in <sup>15</sup>N (P < 0.01). We infer that these fields were treated with chemical fertilizers that are usually less enriched in  $\delta^{15}N$  than organic fertilizers (Choi et al. 2003, Michalski et al. 2015) and are quickly mobilized by plants and microbes. This observation is consistent with land-use maps, which show fields between the villages Kraatz (Kr) and Augustfelde (Au) that were recently cultivated as strips by small farmers, who often applied only small amounts of chemical fertilizers. These fields have recently been merged and are now cultivated by larger farms.

Forested areas were not influenced by fertilization management and exhibited the lowest  $\Delta \delta^{15}$ N and  $\delta^{15}$ N<sub>soil</sub> values. In this case, environmental and structural effects were more apparent across the landscape. We found that forests in which the nitrogen cycle was relatively open  $(\Delta \delta^{15} N \sim 0)$  were more water-use efficient. Intrinsic water-use efficiency is a proxy for the relative amount of carbon assimilated to the amount of water transpired. Plants can manage this tradeoff by assimilating more carbon when stomata are open (i.e., increase the photosynthetic capacity) and by reducing the amount of water loss by closing their stomata (i.e., lowering stomatal conductance). In the forests, we observed an increase in WUE; along with an increase in leaf <sup>15</sup>N, which suggests an increase in leaf N content that can be attributed to an increase in Rubisco content and therefore photosynthetic capacity (Lopes and Araus 2006, Milcu et al. 2014). Furthermore, the isotopic patterns of two smaller forests fragments exhibit greater WUE; and  $\Delta \delta^{15}$ N levels with respect to the larger intact forests that are at least twice the size of the fragments. Fragmentation increases the perimeter to area ratio, which can change biodiversity levels and increase the exposure to surrounding land management (Weathers et al. 2001, Billings and Gaydess 2008, Ziter et al. 2014). Our replication of the different forest fragments is relatively low for conclusive statements, but our finding of plant leaves enriched in  $^{15}N$  relative to the soil  $^{15}N$  values (leading to a  $\Delta\delta^{15}N\sim0$ ) is initial evidence of a change in intrinsic N cycling within the fragments that the trees are able to take advantage of by increasing WUE<sub>i</sub>. The relative open N cycle within the forest fragments manifests either directly from possible fertilization practices that we were not able to detect or indirectly through changes in the local biotic dynamics (e.g., understory plant and microbial community N dynamics).

Grassland patterns were predominantly a function of soil type and land management (i.e., fertilization, grazing livestock, and grass harvested for hay or silage). Grassland plants adjust to the different water regimes, as indicated by the WUE; patterns, but the normalized leaf isotopic values ( $\Delta \delta^{15}$ N) indicate that land management had a larger effect on plant nitrogen balance. Grasslands across the sampling area were primarily located on histosols (i.e., peat soils; Fig. 5) containing high levels of N and were relatively enriched in  $\delta^{15}$ N at our site. However, we also detected enriched  $\delta^{15}N_{soil}$  in grasslands not associated with histosols, especially those close to the village Ottenhagen (Ot). This pattern can be partly attributed to the conversion of grasslands to croplands and vice versa. For example, the most enriched  $\delta^{15}N_{soil}$  for a grassland not growing on histosols had been converted from agriculture just the previous year. Other management prescriptions, such as grazing or fertilization, may also contribute to the enriched <sup>15</sup>N<sub>soil</sub> over time (Frank and Evans 1997, Choi et al. 2003).

### Terrestrial-aquatic linkages

Land-use and kettle hole hydrology played a large role in the  $\delta^{15}N_{sediment}$  isotopic patterns across our site. Sediments of kettle holes from the forest land-use type reflected the dominant beech vegetation type, resulting in significant differences from arable and grassland types. Both arable and grassland kettle hole sediments exhibited a large range in values, and we could not determine the immediate land-management impact on them. Farmers till and fertilize in a close proximity to the kettle holes (often the managed land is <1 m away from the water body) that may result in possible direct transfers of N to the kettle hole through erosion or

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subsurface flow (Lischeid and Kalettka 2012). However, the influence of land-use was readily apparent within sediments of one forest kettle hole lying directly between arable and forested land, which was substantially enriched in <sup>15</sup>N. Interestingly, sediments from kettle holes in the arable and grassland land-use type that were the most enriched in <sup>15</sup>N were also those that are known to dry out during the year. Kettle holes experience dynamic hydrologic conditions including wet-dry cycles (Ireland et al. 2012), and these cycles are known to accelerate organic matter turnover (Fierer and Schimel 2002, Miller et al. 2005, Xiang et al. 2008), which may drive the <sup>15</sup>N enrichment of sediment organic matter in these kettle holes.

In partitioning the potential carbon and nitrogen sources to sediments, we found that plant organic matter from surrounding fields (i.e., crops and grasses) was the dominant input to arable and grassland land-use type kettle holes. Kettle holes within the forest land-use type received a relative equitable contribution from both soil and plant (beech litter). Our partitioning results of the isotopic value of sediments highlight the importance of terrestrial contributions, and while the surrounding terrestrial ecosystems supply a large portion of productivity to water bodies (Cole et al. 2006, Jansson et al. 2008, Berggren et al. 2010), inputs from floating plants, plankton, and encroaching kettle hole semi-aquatic vegetation such as typha and reed plants also occur, which will significantly contribute to the sediment isotopic value.

#### Insights from isoscapes in complex landscapes

We have analyzed over 1500 organic matter samples originating from different plant species, soils, and sediments across a relatively small agricultural landscape divided into at least 80 different management units. This unusually high sample density in a uniform grid was necessary because of the unknown degree of isotopic heterogeneity within a field and across a landscape varying in land-use history and management. Previous isoscapes at all scales have interpolated over at most 500 samples to yield insights into ecological and hydrological spatial patterns (Appendix S1: Fig. S2).

Generating isoscapes across complex landscapes does pose significant challenges regarding scaling and temporal dynamics. Small landscape features (<250 × 250 m, in our case), represented as polygons in Geographical Information Science, are not always accurately accounted for when interpolating across a landscape, yielding a more simplified perspective. In the case of agricultural fields, we may lose resolution because the interpolation across the isoscapes is not constrained by field boundaries. Sample density and grid design are also dependent on the temporal dynamics of the system of interest. For our study, we were careful to observe the different plant sowing times and phenologies, for example, corn and sugar beet were planted in the end of April in contrast to other crops that were planted in the previous winter.

We found land-use and management effects were dominant over the environmental drivers of the landscape and that the heterogeneity in biogeochemistry reflected the local management activity. Land-use practices change with different societal demands and policies necessitating specific field and management information. Our study clearly benefitted from a local research station that serves as a center for outreach and communication to local landowners and managers, providing a wealth of information on changes in land-use and practices for decades. With such a repository, changes in agricultural management, such as applying organic slurries in lieu of chemical fertilizers, to cultural shifts, in our case the reunification of Germany, become tractable. We have shown that isoscapes can be used in complex agricultural landscapes to help visualize multiple sources of biogeochemical, agricultural, and historical information. Future studies may be able to expand upon the initial information we presented to help provide a global view of the different biogeochemical patterns across agricultural landscapes.

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