

## LETTER

# Community scale $^{15}\text{N}$ isoscapes: tracing the spatial impact of an exotic $\text{N}_2$ -fixing invader

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### Abstract

Plant–plant interactions are key processes shaping plant communities, but methods are lacking to accurately capture the spatial dimension of these processes. Isoscapes, i.e. spatially continuous observations of variations in stable isotope ratios, provide innovative methods to trace the spatial dimension of ecological processes at continental to global scales. Herein, we test the usefulness of nitrogen isoscapes ( $\delta^{15}\text{N}$ ) for quantifying alterations in community functioning following exotic plant invasion. Nitrogen introduced by an exotic  $\text{N}_2$ -fixing acacia could be accurately traced through the ecosystem and into the surrounding native vegetation by combining native species foliar  $\delta^{15}\text{N}$  with spatial information regarding plant location using geostatistical methods. The area impacted by N-addition was at least 3.5-fold greater than the physical area covered by the invader. Thus, downscaling isoscapes to the community level opens new frontiers in quantifying the spatial dimension of functional changes associated with invasion and in resolving the spatial component of within-community interactions.

### Keywords

*Acacia longifolia*, ecosystem changes, exotic plant invasion, invasive acacia, isoscape, N-cycle, N-fixation, plant–plant interaction, spatial scale,  $^{15}\text{N}$  stable isotope.

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## INTRODUCTION

New methods are required to accurately capture and record spatially explicit information characterising plant–plant and plant–soil–atmosphere interactions. Spatial dependence and variability of these interacting processes have typically been ignored and experiments and field studies have often been designed with the explicit goal of controlling for or eliminating the inherent spatial dependence in these processes. At the same time, it is being increasingly acknowledged that in order to obtain a complete process-based understanding of ecosystem functioning, the spatial dimension of interactions, alterations and feedbacks within ecosystems should not be neglected (e.g. Rietkerk *et al.* 2002; Beever *et al.* 2006; Brooker *et al.* 2008). In fact, these dependencies may themselves contain crucial information regarding the factors controlling ecosystem functioning. Aspects such as spatial heterogeneity in resource availability, differential resource utilisation by neighbouring species and their interactions (competition and facilitation), are examples of key factors that shape communities and impact ecosystem functioning (Kneitel & Chase 2004; Jones & Callaway 2007). Furthermore, there is a need for an adequate framework for incorporating the spatial component of interactions and feedbacks within plant communities.

Spatio-temporal variations in isotope ratios contain a potential wealth of information regarding ecological processes (West *et al.* 2008), as the stable isotope ratios of environmental substrates are determined by spatially and temporally dynamic biological and chemical processes (West *et al.* 2008; Bowen *et al.* 2009). Recently, due to the expansion of data collection networks combined with increased computing capacity, and further development of geostatistical interpolation methods, interest in explicitly understanding the spatial component of isotope ratio variation has increased. One framework to quantify these variations is the construction of *isoscapes*

(i.e. *isotope landscapes* – spatially continuous observations of spatio-temporal variation in isotope ratios; see West *et al.* (2010) and references therein). To date, the application of isoscapes has typically occurred at landscape, continental or global scales; however, if processes determining isotope ratios vary systematically at smaller spatial scales (e.g. the community level) isoscapes may also give us a useful framework for tracing spatial patterns at more localised scales. Moreover, if isoscapes are computed using the isotope ratios of biological material, they can potentially act as a tracer for the ecological processes responsible for variation in these isotope ratios.

Nitrogen resources are known to exhibit high spatial and temporal variability and are one of the key factors controlling the outcome of plant–plant interactions and triggering changes in community composition (Wilson & Tilman 1991; Clark *et al.* 2007). Specifically, N-source utilisation is a dynamic process, which can vary at small scales even within one plant community (e.g. Stahl *et al.* 2011). Natural abundance foliar  $\delta^{15}\text{N}$  signatures can lend insight into the N-cycle (Dijkstra *et al.* 2008; Kahmen *et al.* 2008) and specifically, into the origin of plant nitrogen (e.g. atmospherically derived vs. soil derived, Högberg 1997) when  $\delta^{15}\text{N}$  signatures of the potential sources are clearly distinguishable from one another (Robinson 2001), and underlying biological, topographical or geographical conditions explain the observed spatial variability (Bedard-Haughn *et al.* 2003). One example is the addition of nitrogen by  $\text{N}_2$ -fixing exotic species during biological invasions, which can dramatically alter ecosystem N-cycling and N use by native species through the introduction of substantial quantities of atmospheric N (Vitousek *et al.* 1987; Lambdon *et al.* 2008). As  $\text{N}_2$ -fixing species typically have greater foliar N content and  $\delta^{15}\text{N}$  signatures closer to the atmospheric value (0‰) than non-fixing plant species,  $\delta^{15}\text{N}$  has the potential to be used as a sensitive tracer of N flow within ecosystems (e.g. Högberg 1997). While much  $^{15}\text{N}$ -natural abundance research has been focused on

quantifying the N-input from nitrogen fixation in terrestrial systems (Virginia *et al.* 1989; Vitousek *et al.* 1989), studies explicitly documenting the spatial extent to which invasive N<sub>2</sub>-fixers influence the N status of the surrounding native vegetation are still lacking.

Herein, we evaluate if spatially resolved measurements of foliar  $\delta^{15}\text{N}$  signatures and N content can be used to trace the flow (input, uptake and utilisation) of atmospheric N<sub>2</sub> fixed by an exotic leguminous tree into a native Mediterranean dune plant community. Accordingly, the overall aim was to test whether an isoscapes framework could be downscaled from the regional scale to provide insights into spatially explicit processes controlling nitrogen cycling at the community level.

## MATERIAL AND METHODS

### Field site and study species

In this study, we used a spatially explicit sampling design in an N-limited sand dune ecosystem in Pinheiro da Cruz, Portugal (N 38°15.2' W 8°45.8'). The dominant species are the invasive non-native leguminous shrub *Acacia longifolia* (Andrews) Willd., the native endemic shrub *Corema album* L., and the native sclerophyllous shrub *Stauracanthus spectabilis* Webb. Both *A. longifolia* and *S. spectabilis* are nitrogen fixers. A previous study indicated that the non-fixing *C. album* accesses atmospheric N<sub>2</sub> derived from the exotic acacia

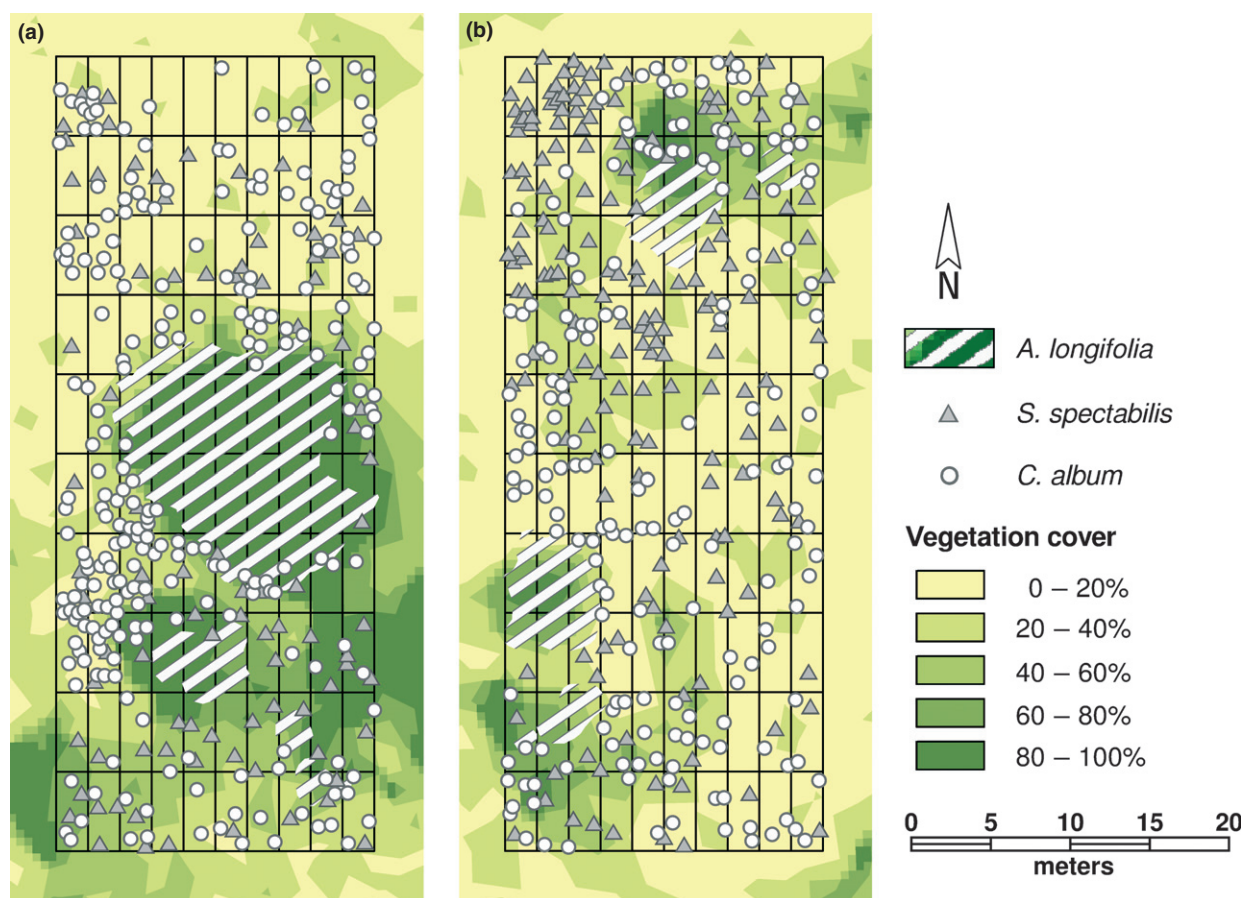
(Hellmann *et al.* 2011). This fact, combined with its high abundance throughout the study area (Fig. 1), made it an ideal monitoring species to evaluate, in a spatially explicit manner, the impact of the N introduced by the invasive species.

### Sampling design

Two 1000 m<sup>2</sup> study areas (20 × 50 m) were established and each divided into one-hundred 2 × 5 m (10 m<sup>2</sup>) subplots (Fig. 1). The study areas were *c.* 100 m distant from one another on a NW–SE transect and differed in dominance of *A. longifolia* (i.e. invasive pressure) with 17% (site 1: high invasive pressure) and 7% (site 2: low invasive pressure) of the plot area covered by *A. longifolia*. Each study area had a 300 m<sup>2</sup> contiguous area where *S. spectabilis* and *C. album* co-occurred in the absence of *A. longifolia*. In each 10 m<sup>2</sup> subplot, current year, fully expanded, sunlit foliage was collected from each individual of the three dominant species and pooled together into one sample per species per subplot.

### Foliage analysis

Foliage samples were oven dried at 65 °C to constant weight and ground to a fine powder using a ball mill (Retsch, Haan, Germany). Nitrogen concentration and  $\delta^{15}\text{N}$  were analysed using an elemental



**Figure 1** Schematic depicting the layout and orientation of the two 1000 m<sup>2</sup> study areas. (a) Site 1 – high invasive pressure: *A. longifolia* covers 17% of the plot area, whereas in (b) site 2 – low invasive pressure: *A. longifolia* covers 7%. The locations of the N<sub>2</sub>-fixing species are indicated: (hatched shading) for the invasive *A. longifolia* and (triangles) for the native *S. spectabilis*. The non-fixing *C. album* (open circles) is broadly distributed throughout both study areas. The background colouring depicts vegetation cover estimated using the normalised difference vegetation index (NDVI) from aerial photographs.

analyser (HEKAtech, Weinberg, Germany) interfaced with a continuous flow stable isotope ratio mass spectrometer (ISOPRIME, GV, Manchester, UK) and measured against ammonium sulphate standard (IAEA.N2). N isotope ratios are presented in  $\delta$  notation:

$$\delta^{15}\text{N}_{\text{sample}} = (\text{R}_{\text{sample}} - \text{R}_{\text{standard}}) / \text{R}_{\text{standard}} \quad (1)$$

where  $\text{R}_{\text{standard}}$  is the  $^{15}\text{N}/^{14}\text{N}$  ratio of atmospheric  $\text{N}_2$  and  $\text{R}_{\text{sample}}$  is the  $^{15}\text{N}/^{14}\text{N}$  ratio of the sample foliage. The repeated measurement precision was 0.2‰.

### Vegetation cover

Vegetation cover was estimated using aerial photographs (Orthophotomaps: spatial resolution = 0.5 m) taken in 2007 (Instituto Geographico, Portugal). The normalised difference vegetation index (NDVI, Rouse *et al.* 1973) was used to estimate vegetation cover and was calculated for each pixel from these images as:

$$\text{NDVI} = (\text{NIR} - \text{VIS}) / (\text{NIR} + \text{VIS}) \quad (2)$$

where VIS and NIR are the spectral reflectance measurements in the visible (0.4–0.7  $\mu\text{m}$ ) and near infrared (0.7–1.1  $\mu\text{m}$ ) respectively. NDVI values were calculated for every pixel (0.5  $\times$  0.5  $\text{m}^2$ ) of the study area and separated by percentage into five ranked categories: 1 = 0–20%, 2 = 20–40%, 3 = 40–60%, 4 = 60–80% and 5 = 80–100% vegetation cover.

### Data analysis

#### Statistical analysis

Comparisons between foliar  $\delta^{15}\text{N}$  signatures and N content between species were made with an ANOVA followed by Tukey's HSD (R version 2.6.2, R Development Core Team, Vienna, Austria, 2008). *C. album* leaf  $\delta^{15}\text{N}$  and N content data were interpolated to a continuous surface area using ordinary kriging (GeoMedia Professional 6.1 with raster extension GeoMedia Grid 6.1, Intergraph Corporation, Huntsville, AL, USA, 2008).

#### Proximity index

To quantify the influence of each of the two  $\text{N}_2$ -fixing species (the invasive *A. longifolia* and the native *S. spectabilis*) on the  $\delta^{15}\text{N}$  signatures and N concentration of the target species (*C. album*) we calculated a proximity index (PI; Ghazoul *et al.* 1998):

$$\text{PI} = \sum_{i=1}^n \frac{1}{Z_i} \quad (3)$$

where  $Z_i$  is the distance in metres from *C. album* to each neighbouring  $\text{N}_2$ -fixing individual (either *A. longifolia* or *S. spectabilis*). This simple index considers both the number of surrounding  $\text{N}_2$ -fixing plants as well as their distances, weighted in favour of the nearest neighbour. Semivariograms from *C. album* leaf N content and  $\delta^{15}\text{N}$  data were used to determine the range (e.g. maximum distance at which significant spatial autocorrelation within the data set is found). The range (20 m) was used to constrain the maximum distance used in calculations of PI. PI with respect to *A. longifolia* was calculated for the entire 1000  $\text{m}^2$  plots. PI with respect to *S. spectabilis* was calculated using a subarea (300  $\text{m}^2$ ) of each plot where *C. album* and *S. spectabilis* co-occurred in the absence of *A. longifolia*.

#### Estimation of the area impacted by N-input from the invasive species

To estimate the area affected by invasion, i.e. the area in which foliar  $\delta^{15}\text{N}$  signatures of the native species were enriched due to input of fixed atmospheric nitrogen, it was necessary to define a threshold  $\delta^{15}\text{N}$  value indicating uptake of fixed atmospheric N. *C. album*  $\delta^{15}\text{N}$  values declined exponentially when plotted against the distance to the *A. longifolia* canopy, approaching an asymptote. This asymptotic value indicates the background  $\delta^{15}\text{N}$  value of *C. album* when it is not impacted by atmospheric N. To determine this background value, we fitted a nonlinear asymptotic model (exponential convergence) to the *C. album*  $\delta^{15}\text{N}$  values with the distance to the *A. longifolia* canopy as the predictor variable:

$$\delta^{15}\text{N}(\text{dist}_{\text{Ac}}) = a + b \times \exp(c \times \text{dist}_{\text{Ac}}) \quad (4)$$

where  $\text{dist}_{\text{Ac}}$  is the distance to the nearest *A. longifolia* canopy and the parameters  $a$ ,  $b$  and  $c$  were fitted iteratively (SPSS version 15.0 for windows, SPSS Inc., Chicago, IL, USA).  $a$  is the value for the lower asymptote,  $b$  is the range of  $\delta^{15}\text{N}$  values and  $c$  is the bending factor. Variation about the asymptote was quite high so we conservatively defined 60% of the asymptotic value to be the cut-off value indicating that *C. album* was taking up fixed atmospheric N, thereby excluding all variance in  $\delta^{15}\text{N}$ , which was not due to *A. longifolia* presence. Accordingly, any *C. album*  $\delta^{15}\text{N}$  value more enriched than the cut-off value indicated the incorporation of fixed atmospheric N. We then used the continuous map of *C. album* foliar  $\delta^{15}\text{N}$  values (obtained by kriging, see above) to compute the plot area impacted by the presence of *A. longifolia*, i.e. the total area where *C. album*  $\delta^{15}\text{N}$  values were above the determined threshold value (GeoMedia Professional 6.1 with raster extension GeoMedia Grid 6.1, Intergraph Corporation, 2008). This estimate of the impacted area was expressed as % of the total plot area.

### RESULTS

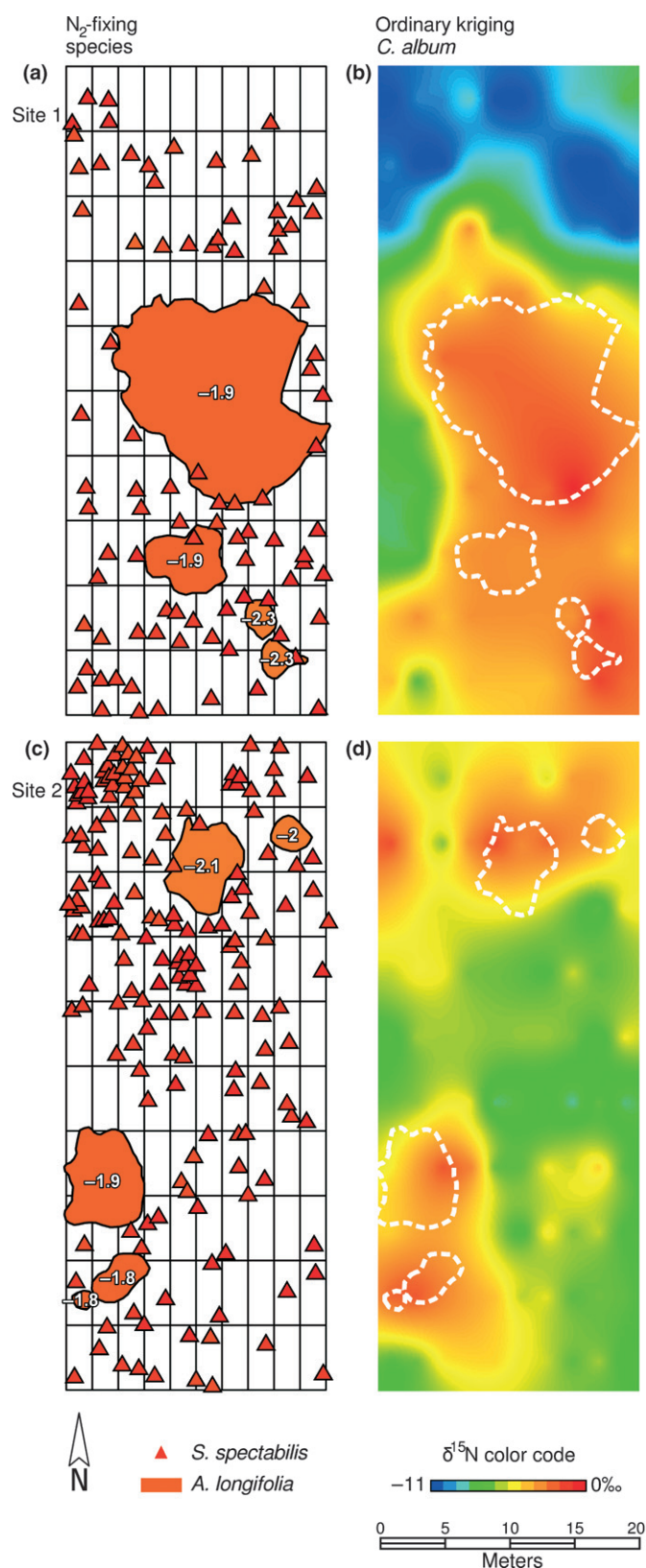
Various characteristics of the selected study area render it an adequate model system to study alteration of N-dynamics following invasion by an  $\text{N}_2$ -fixing species. The native shrub *C. album* was widely distributed throughout the dunes habitat (Fig. 1) and had foliar  $\delta^{15}\text{N}$  values which were strongly depleted (−8‰, Table 1) compared with foliar isotopic compositions of  $\text{N}_2$ -fixing species, which had  $\delta^{15}\text{N}$  signatures nearby to the atmospheric signal of 0‰ (*A. longifolia*, *S. spectabilis*, Table 1). These characteristics made *C. album* a sensitive indicator species for monitoring N introduced to the ecosystem by the invasive  $\text{N}_2$ -fixing *A. longifolia*. Moreover, the presence of the native  $\text{N}_2$ -fixing species, *S. spectabilis*, allowed for the distinction between the effects of a native vs. an exotic legume on community N-dynamics.

Although foliar  $\delta^{15}\text{N}$  signatures close to 0‰ confirmed that both *A. longifolia* and *S. spectabilis* fixed substantial amounts of atmospheric

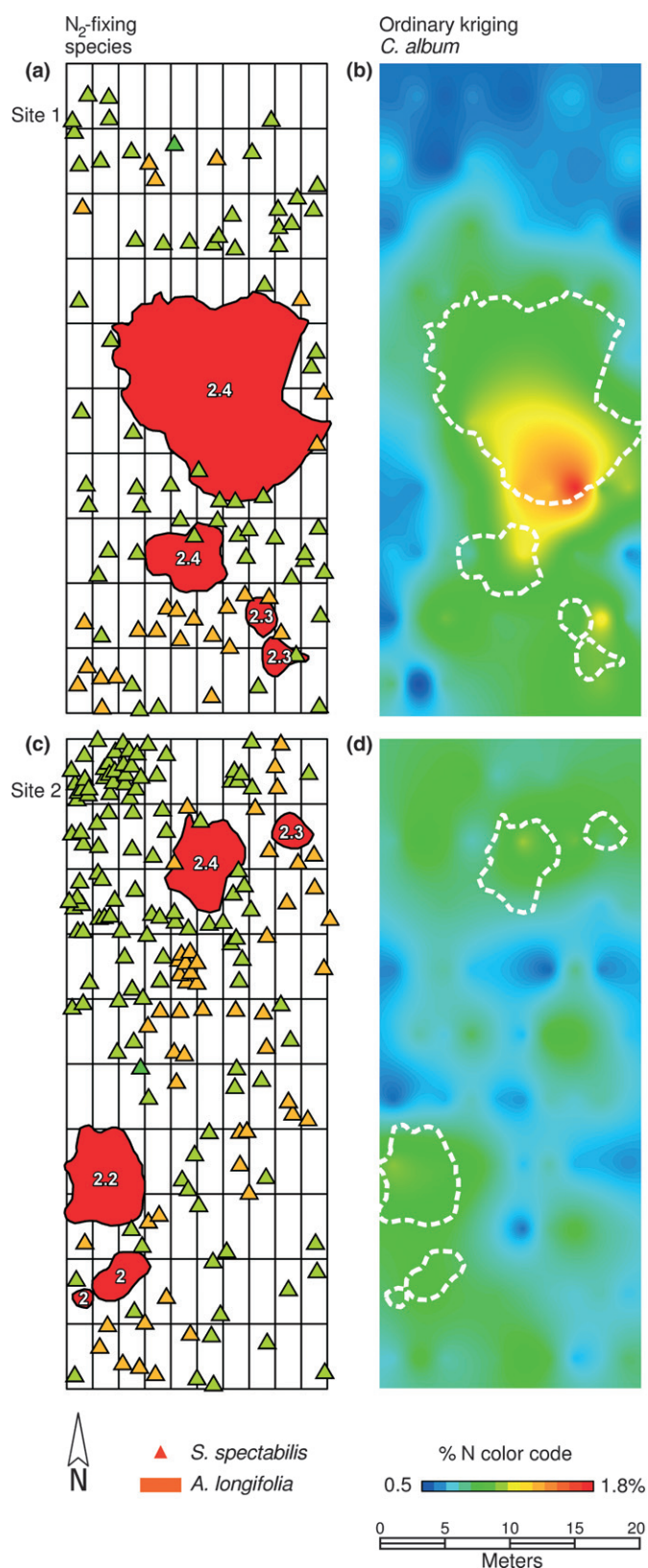
**Table 1** Mean  $\pm$  SD of foliar  $\delta^{15}\text{N}$  and N content for the three studied species

	Foliar $\delta^{15}\text{N}$ (‰)	Foliar N content (%)
<i>Acacia longifolia</i>	−1.98 $\pm$ 0.50*	2.27 $\pm$ 0.30*
<i>Corema album</i>	−7.98 $\pm$ 2.03†	0.69 $\pm$ 0.10†
<i>Stauracanthus spectabilis</i>	−0.66 $\pm$ 0.34‡	1.22 $\pm$ 0.10‡

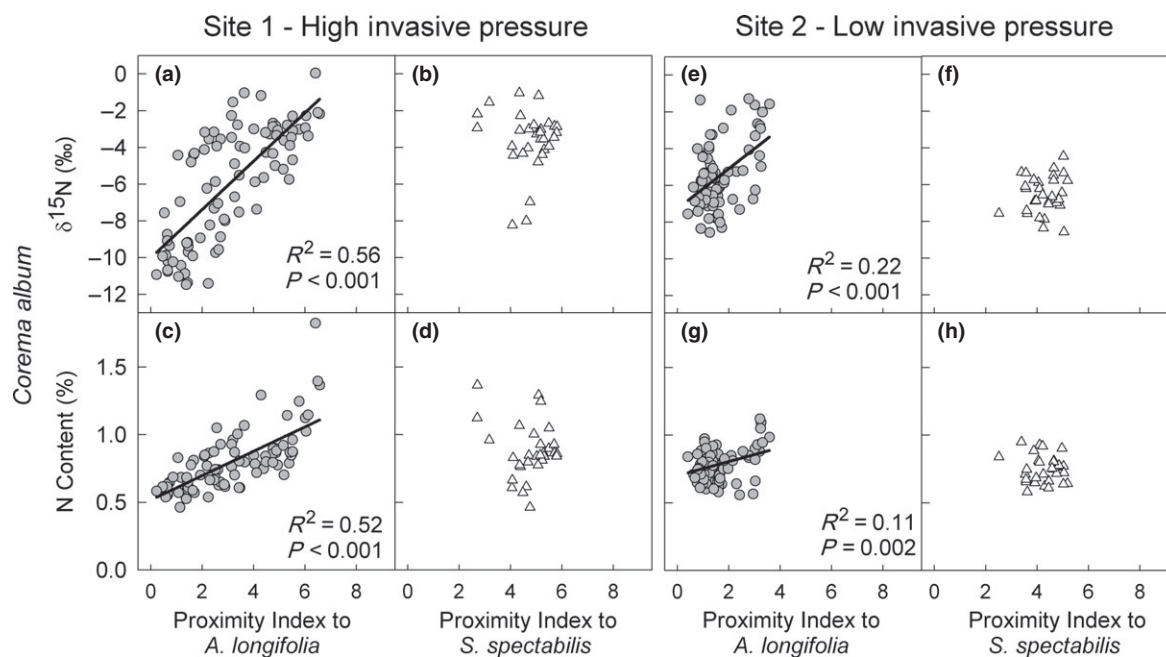
For *C. album* only plants growing in the absence of *A. longifolia* were used (i.e. in the 300  $\text{m}^2$  non-invaded subareas of each plot). Within a parameter different online symbols indicate significant differences of mean values between species ( $P < 0.001$ ); ANOVA followed by Tukey's HSD test.



**Figure 2** Spatially explicit depiction of foliar  $\delta^{15}\text{N}$  signatures of *A. longifolia*, *S. spectabilis* and *C. album* in the two study areas. (a, c) foliar  $\delta^{15}\text{N}$  signatures of the  $\text{N}_2$ -fixing species: (solid area) *A. longifolia* and (triangles) *S. spectabilis*. (b, d) continuous map of *C. album* foliar  $\delta^{15}\text{N}$  with the *A. longifolia* canopies demarcated by a white line.



**Figure 3** Spatially explicit depiction of foliar N content of *A. longifolia*, *S. spectabilis* and *C. album* in the two study areas. (a, c) foliar N content of the  $\text{N}_2$ -fixing species: (solid area) *A. longifolia* and (triangles) *S. spectabilis*. (b, d) continuous map of *C. album* foliar N content with the *A. longifolia* canopies demarcated by a white line.



**Figure 4** Relationships between *C. album* foliar  $\delta^{15}\text{N}$  and N content and proximity indices to the  $\text{N}_2$ -fixing species, (grey circles) *A. longifolia* and (white triangles) *S. spectabilis*. (a–d) Site 1 – high invasion pressure and (e–h) site 2 – low invasion pressure. Proximity indices were calculated with respect to (a, c, e, g) *A. longifolia* and (b, d, f, h) *S. spectabilis*.

nitrogen, foliar N concentrations were higher in the invasive compared to the native legume (Table 1). Further, they exhibited a markedly distinct spatial distribution pattern: *S. spectabilis* formed smaller isolated shrub canopies and was relatively evenly distributed in this open sandy habitat, similar to other native species like *C. album* (Fig. 1). In contrast, the exotic acacia formed larger, monodominant stands with a clumped distribution (Fig. 1). To evaluate whether either  $\text{N}_2$ -fixing species provided an additional nitrogen source for the surrounding non-fixing vegetation, we created continuous maps of *C. album* leaf N content and *C. album* leaf  $\delta^{15}\text{N}$  (isoscapes) for a site with high and low invasive pressure (Figs 2b, d and 3b, d). These maps revealed a substantial discrepancy between the effects of N introduced by the native vs. invasive  $\text{N}_2$ -fixing species. There was a clear spatial dependence between the location of the acacia stands and *C. album*  $\delta^{15}\text{N}$  and foliar N content with *C. album*  $\delta^{15}\text{N}$  approaching 0‰ and foliar N content increasing in the vicinity to *A. longifolia* stands under both high (site 1, Figs 2b and 3b) and low (site 2, Figs 2d and 3d) invasive pressure. In contrast, even though the native  $\text{N}_2$ -fixer *S. spectabilis* had a broad distribution throughout both study sites (Fig. 1), no dependence between the location of *S. spectabilis* and the foliar  $\delta^{15}\text{N}$  or N content of neighbouring *C. album* plants was visually apparent (Figs 2b, d and 3b, d).

To evaluate the significance of the impact of the invader on the native vegetation revealed by isoscapes, we analysed the relationship between a computed proximity index (PI) and *C. album* leaf N content and leaf  $\delta^{15}\text{N}$  (Fig. 4). Significant linear correlations were found for the proximity to *A. longifolia*, indicating that with increasing proximity to the acacia stands (i.e. the exotic nitrogen source) the leaf N content of *C. album* increased (Fig. 4c, g) and leaf  $\delta^{15}\text{N}$  values approached 0‰ (Fig. 4a, e). Moreover, a significant portion of the variability in *C. album*  $\delta^{15}\text{N}$  and N content could be explained by proximity to the exotic invader ( $r^2$  between 0.11 and 0.56 and  $P < 0.01$ ). In contrast, there was no correlation between proximity to *S. spectabilis* and *C. album* foliar N content or  $\delta^{15}\text{N}$  signatures (Fig. 4b, d, f, h). Thus, only the exotic invader significantly altered N availability to native species.

Moreover, the  $\delta^{15}\text{N}$  isotope approach allowed us to quantify the area of the native community affected by the invader: we employed a conservative threshold value of  $\delta^{15}\text{N}$ , which indicated significant uptake of fixed atmospheric N and was determined from an asymptotic regression model of the native species' foliar  $\delta^{15}\text{N}$  vs. distance to the invader. This threshold was applied to the kriging map (Fig. 2b, d) to compute the area significantly impacted by  $\text{N}_2$ -addition (see Material and Methods for details). The impact on the native community was several-fold larger than the physical area occupied by *A. longifolia*: even though the invasive species covered less than 17% of the plot area in site 1, approximately 60% of the area was affected by N-input. Under lower invasive pressure (7% cover, site 2), N-dynamics were altered on 37.1% of the plot area. Accordingly, the area affected by invasion exceeded the area covered by the invader by factors of 3.5 (site 1) and 5 (site 2). Thus, combining community scale isoscapes with an asymptotic regression model enabled the quantification of the area impacted by the invasive  $\text{N}_2$ -fixing species.

## DISCUSSION

This study assesses the applicability of an isoscapes framework to evaluate interactions among species at the community scale. Specifically, we were able to use spatially resolved information of foliar  $\delta^{15}\text{N}$  and N content of an abundant native endemic shrub (*C. album*) to trace atmospheric nitrogen inputs due to  $\text{N}_2$ -fixation by an exotic invasive acacia. This approach presents an improvement over conventional methods investigating plant–plant interactions and ecosystem processes, by adding important information concerning the spatial dimension of these processes.

Traditional approaches, which focus on plant–plant interactions are typically limited to comparing neighbouring individuals (e.g. Ludwig *et al.* 2004; Stahl *et al.* 2011). Indeed, until recently there have been only limited methods for detecting and quantifying processes within ecosystems in a spatially explicit manner and these methods required

exhaustive sampling, which is costly and timeconsuming (Rietkerk *et al.* 2002; Beever *et al.* 2006). Yet such methods are particularly required when studying biological invasions, as they create a local disturbance, thereby disrupting native plant interactions with potential impacts on biogeochemical cycles at micro (e.g. microbial–rhizosphere interaction, Lorenzo *et al.* 2010; Yannarell *et al.* 2011), community (e.g. competition for resources, decline in species richness and diversity and alteration of biogeochemical cycles; Yelenik *et al.* 2004; Gaertner *et al.* 2009; Weidenhamer & Callaway 2010; Rascher *et al.* 2011b), and landscape scales (e.g. altered fire regimes and desertification; Brooks *et al.* 2004; Ravi *et al.* 2009).

Characterisation of plant–plant interactions within a community requires an ecological tracer, which is sensitive to small-scale changes induced locally by individual species, such as N-input from atmospheric N<sub>2</sub>-fixation.  $\delta^{15}\text{N}$  of bulk leaf material of non-fixing native plant species can provide such a tracer, integrating the N-enrichment of the system from an external N-source after disturbance by an invasive N<sub>2</sub>-fixing species. In contrast, soil  $\delta^{15}\text{N}$  provides a less appropriate tracer in sandy soils with very low soil nitrogen content, which makes it typically difficult to follow the flow of N from N<sub>2</sub>-fixing species through the soil to other plant species (Azcón-Aguilar *et al.* 1998; Handley *et al.* 1999). In our case, soil N content outside the acacia canopy and in uninvaded areas was always below the detection limit, preventing the use of soil  $\delta^{15}\text{N}$ . Further, the  $\delta^{15}\text{N}$  signatures of total soil N are not necessarily representative of the  $\delta^{15}\text{N}$  of N-compounds available to plants (Högberg 1997; Pardo *et al.* 2007). The method presented in this study bypasses these issues by getting directly at the question of whether or not atmospheric N<sub>2</sub> fixed by leguminous species is utilised by non-fixing species.

Recently, the isoscapes framework has provided an efficient method for analysing spatial patterns on landscape, regional and global scales (e.g. West *et al.* 2010). The major premise behind isoscapes is that isotopic discriminating processes and the resulting isotopic composition of compounds can be predicted as a function of e.g. time, location or season (Bowen *et al.* 2009). Thus, isoscapes provide a means of interpreting observed isotopic data in terms of spatial patterns within earth systems and can therefore be used to ‘fingerprint’ the origin of geological and biological materials (Bowen *et al.* 2009). In this study, we have adapted the general concept of isoscapes and down-scaled it to the community level within the context of biological invasions (Figs 2 and 3). By doing so, we have demonstrated a method, which allows for an abundant native species, *C. album*, to be used as a ‘biomarker’ (*sensu* Parker *et al.* 1999) to track the influence of an invasive species. Furthermore, this downscaling of the isotopes framework is an innovative way to discern the total impact of an invader as it directly combines the physical extent (i.e. range and abundance) and per-capita effect of the invader into a simple measure, spatially explicit sampling of  $\delta^{15}\text{N}$ .

There is a clear need for early recognition of the ecosystem impacts of exotic species invasions and methods for defining thresholds affecting ecosystem resilience and recovery potential after invasions (Le Maitre *et al.* 2011). Indeed, applying leaf  $\delta^{15}\text{N}$  isoscapes at the community scale combined with a non-linear regression model provided one such tool for quantifying ecosystem transformations by quantifying the spatial impact of the N<sub>2</sub>-fixing invader on the surrounding native plant community and into the non-invaded vegetation. The  $\delta^{15}\text{N}$  isoscapes indicated a clear spatial pattern in the native endemic indicator species, *C. album*, which was significantly correlated with proximity to the exotic N<sub>2</sub>-fixing invader (Figs 2 and 4).

The increase in foliar  $\delta^{15}\text{N}$  of *C. album* with increasing proximity to *A. longifolia* reflects the incorporation of fixed atmospheric N<sub>2</sub> (with  $\delta^{15}\text{N}$  of 0‰) provided by the invader into the native plants’ tissues. The supplementary N presumably enters the system via high inputs of N-rich litter by the N<sub>2</sub>-fixing *A. longifolia* (Marchante *et al.* 2008; Hellmann *et al.* 2011; Morris *et al.* 2011) and apparently, the isotopic signal is largely preserved throughout mineralisation processes probably as it is the dominant mass flow. However, further mechanisms may potentially alter  $\delta^{15}\text{N}$  values such as, higher losses of depleted <sup>15</sup>N under increased nutrient availability, which can result in enrichment of the remaining N pool (e.g. Högberg 1990, 1997) or decreased fractionation during N transfer between mycorrhiza and host plants (Hobbie & Colpaert 2003; Hobbie *et al.* 2008). Furthermore, soil and litter C/N ratio is lower in areas invaded by *A. longifolia* than in non-invaded areas (Marchante *et al.* 2008; Hellmann *et al.* 2011), affecting microbial activity and rates of mineralisation and immobilisation (Dijkstra *et al.* 2008; Marchante *et al.* 2008). However, as the effects of N loss, mycorrhiza-plant N transfer, as well as microbial activity are small (a few per mill, e.g. Hobbie & Colpaert 2003) compared to the effects measured in this study (enrichment of up to 11.5‰), alteration of source  $\delta^{15}\text{N}$  due to input by *A. longifolia* litter can be assumed to be the major cause of  $\delta^{15}\text{N}$  enrichment of *C. album* foliage.

Moreover, the spatial analysis clearly revealed the differential ecological impact of the exotic vs. a native N<sub>2</sub>-fixing species: even though both native and exotic species (*S. spectabilis* and *A. longifolia*, respectively) used atmospheric N<sub>2</sub> as their primary nitrogen source (as indicated by high-leaf N content and  $\delta^{15}\text{N}$  values close to 0‰, Table 1), the native species exhibited no significant influence on the surrounding vegetation. In contrast, the exotic invader caused a significant enrichment in the N status of the native *C. album* (Figs 2, 3 and 4), an effect which extended far beyond the acacia canopy into the non-invaded vegetation, exceeding the covered area several-fold.

These differential impacts on community functioning can be attributed to different functional traits of the exotic invader such as (1) much higher growth rates and leaf turnover (Peperkorn *et al.* 2005; Hellmann *et al.* 2011), (2) differences in phenological cycle with large leaf shedding during the most stressful periods of the year (Otieno *et al.* 2005), (3) differences in biomass decomposition rates (Allison & Vitousek 2004; Yelenik *et al.* 2004) and could further depend on (4) mycorrhizal networks (e.g. Hobbie & Colpaert 2003). Particularly, the large accumulation of N-rich litter under *A. longifolia* canopies results in significant N-inputs due to litter leaching and decomposition, strongly influencing soil N pools and microbial processes (Marchante *et al.* 2008; Lorenzo *et al.* 2010).

These results clearly indicate that the impact of N<sub>2</sub>-fixing invasive species may be underestimated if the effect on the surrounding vegetation is not taken into account and adds another important piece of information for the understanding of the invasive success of N<sub>2</sub>-fixing exotic species, which are among the most problematic invasive species causing large ecological and economical problems worldwide (Mack *et al.* 2000; Liao *et al.* 2008; Gaertner *et al.* 2009). Specifically, Australian acacias have received increasing attention as major or emerging invaders in many parts of the world (Le Maitre *et al.* 2011; Richardson & Rejmánek 2011). Invasive *Acacia* spp. substantially alter soil N properties with long-lasting effects on soil N-cycling and soil microbial communities even after stands of the invader have been cleared (Yelenik *et al.* 2004; Marchante *et al.* 2008; Lorenzo *et al.* 2010). Moreover, previous studies have indicated that the addition of N

through acacia impacts the growth rates and biodiversity of the surrounding native vegetation (Hellmann *et al.* 2011; Rascher *et al.* 2011a) with *C. album* specifically growing more rapidly in proximity to *A. longifolia* (Hellmann *et al.* 2011).

Many invasive species possess novel traits (e.g. high-resource utilisation, water spender) which may disrupt the co-evolved competitive balances within the native plant community (e.g. Morris *et al.* 2011). For example, *A. longifolia* exerts a high-competitive strength due to high-nitrogen uptake efficiency and higher relative growth rate than native competitors under a range of water (Werner *et al.* 2010), light and nutrient (Peperkorn *et al.* 2005) conditions indicating that the N-input in invaded systems may also have a marked self-facilitating effect accelerating the growth and spread of *A. longifolia*. In these nutrient depleted habitats, invasive species cause a local disturbance and alter community functioning (Rascher *et al.* 2011a) as the massive N-input disrupts the natural competitive balance in N-limited systems (Werner *et al.* 2010; Hellmann *et al.* 2011).

The need for better tools to quantify the impact of invasive species has long been recognised (e.g. Parker *et al.* 1999). To our knowledge this is the first report, which accounts for and quantifies the local disturbance caused by an invasive species in a spatially explicit manner. As pointed out above, the area influenced by invasion can go markedly beyond the area occupied by the invader. Quantification of the spatial impacts of invasive species is particularly relevant regarding the risk assessment, eradication and restoration efforts in these systems. Furthermore, since  $\delta^{15}\text{N}$  is a fast, easy and relatively cheap measurement it could feasibly be measured multiple times at the same site to quantify changes in ecosystem processes over time or as a cost-effective means for quantifying the success or failure of eradication and as a way to follow-up after restoration, which is often lacking (Le Maitre *et al.* 2011).

## CONCLUSION

Herein, we showed for the first time that applying  $\delta^{15}\text{N}$  isoscapes at the community scale proved to be a useful framework for quantifying the impact of an invasive  $\text{N}_2$ -fixing species on the surrounding vegetation. Applying isoscapes at the community level demonstrates a significant advance in invasion ecology in that it allows for quantifying the impact of an invader by combining the three fundamental dimensions of invasion (range, abundance and per-capita effect; *sensu* Parker *et al.* 1999) into one easily obtainable measurement. This new tool may potentially bring us significantly further in assessing the ecological impacts of plant invasions at an early invasion state and thus function as an early diagnostic tool for ecological management and restoration in invaded ecosystems, which is urgently needed. Moreover, considering the feasibility and applicability of our approach it may provide a promising tool to identify, quantify and monitor different types of functional plant–plant interactions at a spatially explicit scale. In general, this approach may be applicable to any situation where a local N-source of distinct isotopic signature is affecting surrounding natural systems; for example, high-ammonium input from agricultural systems, intensive farming or industrial pollution. In the case of exotic invasions, carbon and water cycles within the natural community are also markedly affected (e.g. Rascher *et al.* 2011b), which could be traced by  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  reflecting changes in water source utilisation and water use efficiency, respectively. Therefore, exploring the potential of  $\delta^{18}\text{O}$  and  $\delta^{13}\text{C}$  isoscapes for tracing changes in carbon and water cycle

after exotic invasion or changes in community composition could be a promising approach for future investigations. Thus, the community scale isoscape framework opens new frontiers in quantifying the spatial dimension of functional changes in ecosystems.

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## AUTHORSHIP

KGR, CH, and CW conceived of and designed the study. CH and KGR performed research, collected data, analysed output data, produced spatial plots, statistics and modelling under supervision of CW. CM contributed to discussions of the results. KGR wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

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