Article

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Soil conditions and landscape factors dictate macro-snail communities of newly created grasslands on restored landfill sites in the UK

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Running Title: Land snails on restored landfill sites

Abstract

Though restored landfill sites provide habitat for a number of taxa, their potential for land snail remains unexplored. In this study, large-sized land snails (> 5 mm) were surveyed using transect sampling on nine restored landfill sites and nine corresponding nature sites in the East Midlands region of the UK during 2008. The effect of restoration was investigated by examining land snail species composition, richness, and diversity (Shannon Weiner index) in relation to habitat and landscape structure. Thirteen macro-snail species were found in total and rarefied species richness and diversity on restored landfill sites was not found to be statistically different to that of reference sites. One third of the snail species, comprising 30% of total abundance, found in the restored landfill sites were non-native species introduced to the UK. Soil electrical conductivity was the strongest predictor for richness and diversity of land snails. Road density was found to have a positive influence on snail species diversity. Given the high percentage of introduced species detected further research is needed in terms of the management implications of restored landfill sites and the dynamics of native versus non-native species.

Keywords: land snail, waste ground, restoration, grassland, landfill.

Introduction

Land snail populations are considered to be relatively stable (Lydeard et al. 2004), however, the abundance of some grassland snail species may have changed mainly due to intensive management practices coupled with habitat loss (Kerney 1999; Martin and Sommer 2004a; Stoll et al. 2009). Many invertebrate species of conservation importance are established on brownfield sites such as landfill (Judd and Mason 1995; Rahman et al. 2015; Tarrant et al. 2013). Therefore, the process of habitat restoration could be important for the enhancement of other invertebrate species such as land snails which may have been declined locally or regionally such as *Arianta arbustorum* and *Candidula gigaxii* (Seddon et al. 2014). In England and Wales there are approximately 2,200 landfill sites covering *ca.* 28,000 ha (EA 2006) which is large area of land with conservation potential, but which remains largely unexplored for invertebrates such as land snails.

As detritivores land snails are an integral part of ecosystems (Caldwell 1993; Kappes et al. 2007) including playing vital roles as food for higher trophic levels (Eeva et al. 2010). However, community composition of grassland snails is influenced by habitat variation from the local to landscape-scale (Magnin et al. 1995; Martin and Sommer 2004a, Boschi 2007). Traditionally, calcium is considered as limiting factor for colonisation and distribution of land snail species richness and density (Ondina et al. 1998) although it might be substituted with soil pH or soil buffer system (Cameron et al. 1980; Kappes and Topp 2014). Less often, soil moisture and litter depth are used to explain any such differences (Jurickovά et al. 2008; Hettenbergerovά et al. 2013). Distance to the habitat border and land use within a radius of 1 km can predict snail assemblages in stable habitats such as old forests (Kappes et al. 2009b; Kappes et al.

2011) although connectivity at the landscape scale may have an even larger influence on land snail colonisation of newly created habitats (Kappes et al. 2009a; Stoll et al. 2009, Knop et al. 2011). Recognising critical local and landscape-level factors is important in developing effective conservation strategies for newly created habitats. However, there is a lack of knowledge of which factors are the most important (Ondina et al. 1998). In forests Martin and Sommer (2004b) noticed that moisture availability can somewhat shadow the expected patterns from pH in a set of different forests, whilst Kappes et al. (2007) found that enhanced habitat quality of coarse woody debris increased snail richness and densities over expectations from soil pH alone in a paired sampling approach. In contrast very little research has been conducted on factors affecting assemblages of snail species on restored grasslands and there is also a lack of baseline information on the diversity of snails of restored landfill sites (Wheater and Cullen 1997; Watters et al. 2005). Detailed studies of the relationship between the composition of land snail communities and their local and landscape habitat can provide an understanding of the impacts of restoration and determine the role of newly created grassland habitats in supporting land snail species. Knop et al. (2011) stated that structural connectivity of grassland habitats is important for increasing the restoration success for snails. We therefore hypothesised that macro-snail species richness and diversity are: 1) positively related with management (in terms of mowing), seeding and the age of the sites, and 2) different local habitat and landscape variables may influence the establishment of snail species on restored landfill sites. The present research aims to reveal any distribution patterns of land snail species and to investigate which local and landscape factors control their diversity and abundance on newly created grassland of restored landfill sites.

Methods

Study sites

The study was conducted in the East Midlands region of the UK in the counties of Northamptonshire, Bedfordshire, Warwickshire and Buckinghamshire. Nine restored landfill sites (hereafter LF) were selected randomly from a set of 42 known LF site in this region (Fig. 1). The sites had similar characteristics and were representative of LF sites within the region. In order to provide a comparison, nine reference sites (hereafter RF) were selected which were the closest recognized protected grassland sites for their nature conservation value, being designated as either Local Nature Reserves (LNRs) or Sites of Special Scientific Interest (SSSIs) for their local or national special natural interest respectively. The RF sites were spatially close enough (mean distance = 4.5 ± 3.5 km, range = 1.3–11.8 km) to the LF sites so that they experience similar physiography, climate, soil and land use history (see details in Rahman et al. 2015). Six of the restored landfill sites were managed by mowing during the late summer and three sites had no mowing or grazing regime (Table 1).

Sampling method

All land snails along two randomly selected transects of 100 m long x 2 m wide crossing each other at the approximate centre point of the site, were collected by hand from vegetation, soil and sifted ground litter. Surveys were conducted three times from April-September with a regular interval (*ca.* 10 weeks intervals) during 2008 to provide a sampling regime with a good coverage in spring-summer. Each transect was searched extensively by two people (approximately 30-60 minutes). In this study, snails on the soil surface (i.e. micro-snails and also some small size juveniles which were less 5mm) were not considered. However, some small snail species *Vitrea crystallina*, *Euconulus fulvus*, *Nesovitrea hammonis* and *Vitrina pellucida* were excluded from further analysis except listing of species as few individuals were found. Our sampling efforts were restricted to spring-summer and we did not include snails of soil as it was not our objective to obtain a full list of land snail species for each of these sites but to use standardised sampling as a means of comparison between LF and RF sites. Both live and dead snails were collected and all snails were preserved for further identification following the method of Kerney and Cameron (1979). Snails were cleaned under running water, and then transferred to 70% alcohol and stored for later identification using Kerney and Cameron (1979) and Cameron (2003). Nomenclature follows Beedham (1972). We also classified snails as native or introduced in our study area based on Kerney (1999) though some of those introduced are quite ancient, dating from Roman times.

Local and landscape variables

From each of the LF and RF sites, five soil samples from a depth down to 10 cm were collected for soil analysis from random locations along the transects. Soil moisture content (%), electrical conductivity (microSiemens per centimetre, (µS/cm) which indicates the amount of dissolved minerals present in the soil), stone content (%), litter depth (mm) and pH were determined following Rowell (1994) (Table 2). We used percentage of total area of non-crop features such as grassland, woodland, and road networks as an indicator of the amount and diversity of perennial habitats in the surrounding landscape derived from Land Cover Map 2000 (25 m × 25 m resolution)

(LCM 2000). Percentage of the total area of grassland and woodland and road networks (since road networks may also indicate urban pressure), within a 1 km radius zone of each site's margins were determined using a Geographical Information System to measure for potential landscape-scale effects (ESRI 1999; Table 2).

Statistical analysis

We used rarefied number of species for a total of two individual samples using the R package "vegan" (Oksanen et al. 2013; R Development Core Team 2013) and the Shannon diversity index for analysis as we found high differences in number of individual snails in terms of richness between LF and RF sites which been tested with Generalized Linear Models. Land snail species composition and their abundance between site types (LF and RF sites) were expressed by non-metric multidimensional scaling (NMDS) using Euclidean distance, again using the package "vegan" in R. We log-transformed number of recorded individuals because abundance also greatly varied between sites (Table 1). We examined similarities of macro-snail species composition of the two site types by Analysis of Similarities (ANOSIM) using 999 permutations. Generalised Linear Models (GLMs) were constructed to examine the effects of management, method of site colonization (seeded or natural), age of the LF sites affecting richness and diversity. This particular analysis is limited to only LF sites as the method of colonization and age of RF sites were unknown. Furthermore, separate independent models were also built for richness and diversity of both LF and RF sites taking into account local factors (site type, moisture content, soil electrical conductivity, pH, and litter depth) and landscape-scale parameters (percentage area of grasslands, woodlands and road networks) separately assuming a Poisson and inverse Gaussian distribution for richness and diversity respectively as richness are count data and diversity are discrete data. We compared candidate models with null models which is intercept only model using the Akaike Information Criterion (AIC), one of the most powerful approaches for model selection from a set of alternative plausible models and which solves the problems of stepwise model selection and also corrected for small sample sizes (Burnham and Anderson 2002). Model selection and multi-model inference were implemented in R using the "MuMIn" package (Barton 2013). Akaike weights were assessed to find best supported models. The top-ranked models had an Akaike weight >0.05; we used multi-model inference to compute the model-averaged estimates of the explanatory variable and 95% confidence interval (Burnham and Anderson 2002). A 95% confidence interval excluding 0 indicated that the response variable varied with the explanatory variable of interest (Burnham and Anderson 2002).

Results

Snail composition and effects of seeding, management, age

A total of 13 macro-snail species (10 species in LF and 10 species in RF sites) with 838 individuals (681 in LF and 157 in RF sites) were recorded from nine LF sites and their corresponding RF sites. Seven species were found both on LF and RF sites, while three introduced species were found exclusively in LF site and three native species found exclusively on RF sites (Table 3).

The NMDS ordination of macro-snail composition showed a clear separation between the LF and RF sites along the horizontal and vertical axis primarily due to the high proportion of introduced species in LF sites (ANOSIM test R=0.25, P=0.01), though there is clear separation of three of the LF sites along the first axis which indicates that those sites share few snail species among themselves (Fig. 2). The RF sites showed low variance in spread which indicated a higher similarity to one another. There was also no significant difference in mean rarefied species richness per site (P=0.32) and diversity (P=0.13) between LF and RF sites but there was a significant difference in species richness (P=0.03) (Table 3). Only the model incorporating seeding variable is most parsimonious for snail species richness. However, we did not find any statistical support for effect of seeding on species richness of native or introduced plant species. However, we found seeding has a positive significant effect on *Candidula intersecta* which is an introduced species (t=4.05, p=0.009). None of the models incorporating management or age of the LF sites were found to be parsimonious (AIC >2) for snail species richness and diversity (Table 4). We did not find any particular species having any effect due to management (mowing) on restored landfill sites.

Local and landscape factors on snail richness and diversity of both LF and RF sites

GLM analysis and model selections suggested that land snail species richness and diversity were related to both local and landscape factors (Table 5). Both richness and diversity models that included soil conductivity had the highest support (Akaike weight of 0.22 for richness and 0.34 for diversity). However, the model containing soil conductivity and site type was also an equally parsimonious model as the moisture content model for snail species richness (Table 5).

At a landscape scale, the snail species richness model that considered a fixed effect of road density had the highest support (Akaike weight 0.19) and the richness model containing an additive effect of road density and woodland, road density and grassland on surrounding landscape were equally parsimonious. The diversity model containing only road density had the highest support (Akaike weight 0.69) (Table 5).

We found a positive effect of conductivity on both richness and diversity of snail species but found no evidence of an effect of soil moisture and litter depth on both species richness and diversity. There was a negative effect of road density on the Shannon diversity index and we found no evidence of an effect of grassland and woodland on either snail richness or diversity (Table 6).

Discussion

Snail composition and effects of seeding, management, age

Though we only included macro-snails, the land snail species in the present study represent approximately 15% of the total land snail species of the UK suggesting that restored LF sites has potential as habitat for a significant fraction of the species of this taxon. These snails in turn can have roles in the processes of succession and nutrient cycling of these newly created grasslands (Holland et al. 2007). However, one third of the snail species, comprising 30% of total abundance, found in the LF sites were introduced species to the UK. Such non-native land snail species may cause major changes to these novel ecosystems by supressing native species. Further research is needed to assess whether these European 'exotics' have any impact or not (but see Holland et al. 2007).

We did not found any variation in snail species richness and diversity due to site management, age of the sites, or whether sites were seeded or not. However, seeding may potentially affect land snail densities through enhancing vegetation cover which may reduce extreme and abrupt changes in the microclimatic conditions, such as high temperatures. In previous studies grasslands subjected to constant grazing had decreased land snail diversity and abundance indicating that high grazing pressure may be detrimental to snails (Cameron and Morgan-Huws 1975; Labaune and Magnin 2002; Ruesink 1995). In our landfill sites mowing is restricted to late summer which might be a reason for no negative effects being detected. However, this may also be due to the limited range of ages (4-15 years), related to the time available for establishment or attaining stability within the biotic and abiotic components of the site.

Effect of local and landscape factors on richness and diversity of land snail

The conductivity in the study sites ranged from 12-110 μScm^{-1} and pH ranged from 4.2-7.8. A positive relation between electrical soil conductivity and land snail species richness and diversity indicates the gradient of different available minerals is important factors for snail composition though we do not know which minerals are most important in our samples. We recommend further research should be conducted to determine which minerals could be important factors as other researcher have found effects of different minerals (Ondina et al. 1998; Juricková et al. 2008; Horsák 2004).
The results from this study confirmed that land snail richness was structured by a gradient of woodland in the vicinity though we did not found strong support for this (Table 6). Small grassland patches such as our recreated grassland on restored landfill sites may benefit from the presence of woodlands in the vicinity for land snail colonisation to take place (Labaune and Magnin 2002) particularly introduced generalist species such as *Candidula intersecta* and *Cernuella virgate.* Sites near to woodland had higher land snail richness than those of open areas, which indicates the community is

enriched due to dispersal if distances to sources of immigration are not too far (Cameron et al. 1980; Magnin et al. 1995). A gradient of road density was also found to be one of the strong predictors for land snail species diversity and some species such as *Trichia striolata* (t=2.14, P=0.03). Many introduced land snails are associated with human habitation and roads or anthropogenic disturbances, therefore the positive influence of roads found on Shannon diversity in this study may reflect greater opportunities for both native (t=3.70, P=0.002) and invasive species but did not find any relationship with invasive species (t=1.66, P=0.12. Though most land snails have a restricted active dispersal capability, passive transport allows them to colonise new habitats (Dörge et al. 1999).

In conclusion, the creation (or re-creation) of grassland habitat within fragmented landscapes has potential to enhance biodiversity conservation. However, the high proportion of non-native snail species found in grasslands in this study presents an interesting opportunity to further research the interactions between native and non-native species in terms of their ecology and management.

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Disclosure statement

There are no potential conflicts of interest of anybody or organisation relevant to this research.

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Tables:

Table 1: Abundance (abund), richness and diversity (divers) of native and introduced snail species in restored landfill (LF). Values in parentheses are their corresponding reference (RF) sites parameters.

			Native Species			Introduced Species		
	Seeded	Manage	abund	richness	divers	abund	richness	divers
1	Yes	Yes	18 (12)	3 (3)	0.65 (1.08)	16 (9)	2 (2)	0.69 (0.69)
2	Yes	Yes	10 (48)	2 (4)	0.69 (0.64)	16 (0)	2 (0)	0.69 (0.00)
3	Yes	Yes	56 (1)	2 (1)	0.21 (0.00)	4 (1)	1 (1)	0.00 (0.00)
4	Yes	No	66 (0)	1 (0)	0.00 (0.00)	1 (0)	1 (0)	0.00 (0.00)
5	No	No	39 (2)	2 (1)	0.12 (0.00)	39 (3)	5 (2)	1.14 (0.64)
6	No	No	12 (0)	4 (0)	0.98 (0.00)	0 (0)	0 (0)	0.00 (0.00)
7	No	Yes	47 (53)	2 (4)	0.29 (0.82)	20 (0)	2 (0)	0.20 (0.00)
8	Yes	Yes	227 (8)	4 (2)	0.21 (0.56)	101 (1)	3 (1)	0.73 (0.00)
9	Yes	Yes	3 (19)	2 (3)	0.64 (0.82)	6 (0)	2 (0)	0.45 (0.00)

Table 2: Mean, Median values with their Minimum (Min) and Maximum (Max) of studied local and landscape variables

Parameters	LF Sites (N=9)				RF sites (N=9)			
	Mean	Median	Min	Max	Mean	Median	Min	Max
Moisture content (%)	29.93	30.89	20.46	40.46	39.07	35.72	29.96	53.04
pH	7.25	7.06	6.42	8.01	7.19	6.99	6.39	8.12
Soil Electrical Conductivity	52.57	48.96	19.46	98.14	41.39	29.12	5.75	104.32
Litter depth (mm)	7.44	5.80	0.80	17.40	5.91	4.00	3.60	10.40
Stone content (%)	6.30	7.21	0.49	12.71	0.81	0.18	0.00	2.55
Woodland (% area)	5.70	2.93	0.41	19.80	3.29	1.92	0.00	11.53
Grassland (% area)	17.98	18.34	7.47	32.13	22.02	16.27	4.83	71.57
Road network (% area)	6.73	5.73	2.44	14.47	8.08	6.75	1.14	19.36

Table 3: List and number of land snail species found only landfill sites (LF), only reference sites (RF) and species found on both LF and RF sites. Numbers in parentheses are number of sites, ± denotes standard error.

Species	LF sites	RF sites
Arianta arbustorum	0	2 (1)
Cepaea hortensis	10 (6)	5 (3)
Cepaea nemoralis	5 (2)	2 (1)
Monacha cantiana	445 (8)	103 (7)
Oxychilus cellarius	0	6 (1)
Succinea putris	0	3 (1)
Trichia hispida	11(4)	2 (2)
Trichia striolata	7 (2)	20 (3)
*Candidula gigaxii**	2 (1)	0
*Candidula intersecta**	119 (6)	6 (3)
*Cernuella virgata**	77 (8)	8 (3)
*Helix aspersa**	4 (2)	0
*Helix pomatia**	1 (1)	0
Euconulus fulvus	1(1)	0
Nesovitrea hammonis	1 (1)	1 (1)
Vitrea crystallina	0	2 (2)
Vitrina pellucida	1 (1)	0
Mean macro-snail species richness per site	4.78 ± 0.54	3.00 ± 0.65
Mean rarefied macro-snail species richness per site	1.52 ± 0.08	1.28 ± 0.24
Mean Shannon diversity of macro-snails	0.95 ± 0.14	0.72 ± 0.16

* Introduced species. Source: Kerney (1999)

Table 4: Model selection results for richness and diversity with seeded sites, management and age of the LF sites. K = No. of paramters, AICc = Akaike's information criterion corrected for small smaple sizes, ΔAICc = AICc relative to the top most model, w_i = AICc model weight

Models	K	AICc	ΔAICc	w_i
Richness				
Null model	1	37.3	0.00	0.51
Seeded	2	38.9	1.64	0.22
Age	2	40.5	3.21	0.10
Management	2	40.7	3.37	0.90
Diversity				
Null model	2	-42.9	0.00	0.74
Management	3	-39.2	3.69	0.12

Table 5: Model selection results for richness, diversity with their local and landscape parameters. (Parameters: Rdnet=Road network). K = No. of paramters, AICc = Akaike's information criterion corrected for small sample sizes, ΔAICc = AICc relative to the top most model, w_i = AICc model weight

Models	K	AICc	ΔAICc	w_i
Richness				
Local variables				
Conductivity + Site type	3	72.9	0.00	0.22
Conductivity	2	73.0	0.08	0.21
Moisture content	3	74.2	1.31	0.11
Landscape variables				
Rdnet	2	75.3	0.00	0.19
Rdnet + Woodland	3	76.0	0.67	0.14
Null model	1	76.4	1.09	0.11
Rdnet + Grassland	3	76.7	1.36	0.10
Diversity				
Local variables				
Conductivity	4	-89.4	0.00	0.34
Null model	3	-88.0	1.43	0.16
Conductivity + Site type	5	-86.9	2.44	0.10

Landscape variables				
Rdnet	3	-91.9	0.00	0.69
Rdnet + Grassland	4	-88.8	3.09	0.15
Rdnet + Woodland	4	-88.4	3.48	0.12

Table 6: The explanatory variable selected in GLM for occurrence of and snail species richness and Shannon-diversity on restored landfill sites and reference sites. Numbers in bold shows response variable varied with the explanatory variable. Est.= Parameter Estimates, SE = Standard Error, CI = Confidence Intervals.

	Variables	Est.	SE	Lower 95% CI	Upper 95% CI
Richness	Management	0.08	0.32	-0.68	0.83
	Seeding	-0.42	0.31	-1.17	0.33
	Age	0.15	0.33	-0.62	0.93
	Site type	-0.44	0.26	-0.99	0.11
	Conductivity	**0.57**	**0.25**	**0.04**	**1.11**
	Moisture content	-0.39	0.31	-1.05	0.26
	Road network	0.49	0.25	-0.04	1.03
	Grassland	0.30	0.24	-0.21	0.82
	Woodland	0.38	0.25	-0.15	0.92
Diversity	Management	0.01	0.01	-0.02	0.03
	Conductivity	**0.02**	**0.01**	**0.00**	**0.03**
	Site type	-0.01	0.01	-0.02	0.01
	Road network	**0.02**	**0.01**	**0.01**	**0.04**
	Grassland	0.01	0.01	-0.01	0.02
	Woodland	0.00	0.01	-0.01	0.01

Figure Legend

Fig. 1: Location of studied restored landfill sites (triangles) and their corresponding reference sites (black dots). Numbers on the map correspond to descriptions of landfill sites in Table 1.

Fig. 2. NMDS ordination of snail species composition and their abundance on LF and RF sites. Two-dimensions uses, S-stress=0.08. Nine LF and RF site denote LF(1-9) and RF(1-9) respectively.

Figures:

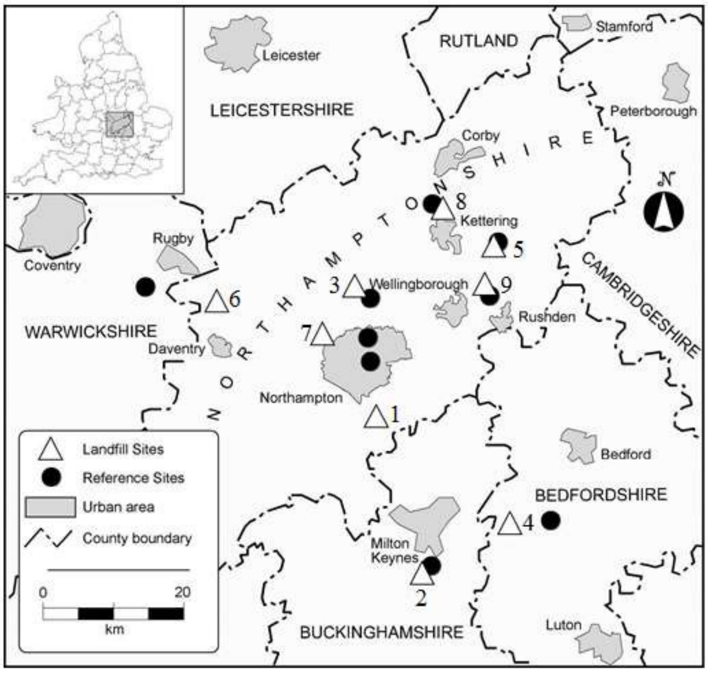

Fig. 1

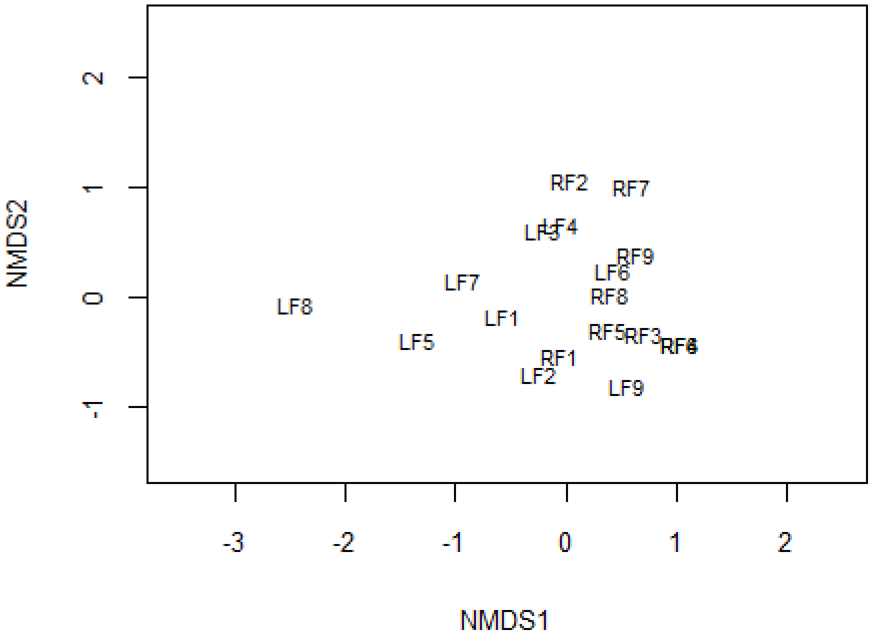

Fig. 2