No need for grazing exclusion – Sheep grazing supports grassland recovery even from the early successional stages

Keine Notwendigkeit für einen Beweidungsausschluss – Schafbeweidung unterstützt die Renaturierung von Grasland, sogar von frühen Sukzessionsstadien an

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Abstract

Availability and dispersal of target plant propagules and applied management techniques can considerably affect the success of grassland restoration. In our study we explored the effect of sheep grazing on plant species composition of an early staged recovering grassland, which developed on newly created soil surfaces. We recorded the presence and cover of vascular plant species in 17 grazed and 6 ungrazed plots during three consecutive years after the restoration of a landfill in southern Italy. A DCA ordination based on species percentage cover was calculated to assess the species composition of the plots in the three years. Plant assemblages were compared to adjacent reference grassland in terms of species composition and cover of functional groups based on their role (i.e. target species or weeds) and their seed dispersal potential (i.e. high or low epizoochorous ranking index). For each parameter, Relative Response Indices (RRIs) were calculated to assess the relationship between the vegetation characteristics of the restored areas and the reference grassland. The DCA ordination of plant communities in the restored area revealed gradients of increasing similarity to reference grassland as a function of successional age and grazing. For most of the considered vegetation characteristics, RRIs in restored grassland became more similar to the reference grassland with increasing successional age and under grazed conditions. Besides underlining the role of passive restoration in supporting effective grassland recovery, our results revealed that extensive sheep grazing even from the early successional stages can improve target species dispersal and establishment, and enhance grassland restoration. Our results suggest that grazing can improve the feasibility and sustainability of restoration projects by saving costs of fence installation and providing forage for local animal husbandry.

Keywords: calcareous dry grassland, epizoochory, landfill, passive restoration, pasture, plant dispersal, semi-natural grassland

Erweiterte deutsche Zusammenfassung am Ende des Artikels
1. Introduction

Extensively managed semi-natural pastures are among the most species-rich habitat types in Europe (Wilson et al. 2012, Roleček et al. 2019), and have an exceptional importance in preserving biodiversity in agricultural landscapes (WallisDeVries et al. 1998, Dengler et al. 2014). Degradation and loss of grassland habitats resulted in a considerable decrease of ecosystem functions and services, and a loss of biodiversity at both local and landscape scales (Papanastasis et al. 2015, Sutcliffe et al. 2015). Thus, the recovery of grassland biodiversity became a top priority in both scientific research and practice (Bakker & Berendse 1999, Habel et al. 2013). As successful restoration of plant communities mostly depends on the establishment of target species, availability and dispersal of plant propagules may represent a bottleneck for successful restoration (Bakker & Berendse 1999, Wolters et al. 2005, Ozinga et al. 2009). Adjacent grasslands can act as important source habitats from which target species colonize after restoration (Bakker & Berendse 1999, Öster et al. 2009, Winsa et al. 2015). However, proximity to the propagule source in its own does not guarantee a fast grassland recovery in case of seed dispersal limitation (Ozinga et al. 2009, Deak et al. 2015). It has been suggested that the loss or decline in long-distance seed dispersal vectors could be a major factor negatively influencing plant species richness in current landscapes (Poschlod et al. 1998).

Dispersal limitation may heavily affect the establishment of grassland plant species, which generally have a poor dispersal ability (Mortimer et al. 1998). Active vectors are needed to ensure their long distance dispersal (Poschlod et al. 1998). For most temperate grassland species, ungulates may provide the means for this critical mobile stage (Stiles 2000). When both propagules of target species and dispersal vectors are present in the surrounding matrix, establishment of target species can be faster in the recovering habitats (Valkó et al. 2017). In such cases, passive restoration can rely on the locally available propagule sources (Prach & Rehounková 2008, Török et al. 2011, Albert et al. 2014), offering a cost-effective way of recovery. In habitats with high productivity, medium intensity of grazing may support species diversity by suppressing the dominant competitor species and creating gaps in the dense sward, thereby facilitating the establishment of subordinate species (Díaz et al. 2001, Tarhouni et al. 2015, Török et al. 2016, Bocht et al. 2018). Livestock may serve as effective zoochorous dispersal vectors, and hence their presence can increase plant species richness (Fischer et al. 1996, Rosenthal et al. 2012, Winsa et al. 2015). In grasslands with a long history of continuous grazing management, species adapted to zoochorous dispersal are well represented (Purschke et al. 2012). In particular, studies on epizoochory by sheep have shown the great potential of animal vectors in terms of dispersal distance, abundance and diversity of transported seeds (Fischer et al. 1996, Moussie et al. 2005, Manzano & Malo 2006, Wessels et al. 2008). Due to their curly and greasy hair, sheep epizoochory is possible for most if not all grassland species (Moussie et al. 2005). Besides epizoochory, sheep are also important endozoochorous dispersal vectors: Moussie et al. (2005) estimated that one sheep can disperse approximately 40,000 seeds per year via endozoochory. Thus, sheep grazing can be especially valuable for the dispersal of target grassland species, which is particularly important for conservation and restoration purposes (Wessels et al. 2008).

In our study we investigated the spontaneous vegetation recovery on newly created open soil surfaces in grazed and ungrazed conditions. Since the restored area was adjacent to semi-natural grasslands, we were able to define the reference state of grassland recovery and
to compare vegetation characteristics of the recovering grassland to those of reference grassland. High similarity to reference grassland was considered as the restoration target in terms of species composition and cover of target and weedy species. Cover of species with high and low epizoochory ranking index (ERI; HINTZE et al. 2013) was used to assess the effect of sheep grazing on seed dispersal. With regard to the restored area, we hypothesised that: (1) the species pool, especially the cover of target and weedy species and the cover of epizoochorous species, becomes similar to the reference grassland with successional time, and (2) grazing facilitated this process.

2. Study area

The restored area is located on a hilltop of Alta Murgia (600 m a.s.l.; central coordinates: 41.0851° N; 16.1023° E), a calcareous plateau in southeast Italy (Fig. 1). It is included within the Natura 2000 site “Murgia Alta” and the National Park “Alta Murgia”. Ranging from 300 to 700 m a.s.l., Alta Murgia is primarily characterized by its compact platform of Cretaceous limestone, with very shallow and rocky soils and the total lack of surface water courses. The climate is sub-Mediterranean, with mean temperatures from 7 °C in January to 25 °C in July and August, and precipitation mostly in autumn and winter (600 mm/yr on average), with meso-Mediterranean thermotype, ombrotype from dry to sub-humid, and growing season bound by both winter temperature and summer drought (MAIROTA et al. 2013).

![Fig. 1](image)

Fig. 1. Location of the study area and distribution of sampling units.

Abb. 1. Lage des Untersuchungsgebiets und räumliche Verteilung der erhobenen Flächen.
With the exception of a few patches of downy oak (*Quercus pubescens* s.l.) woodland and Aleppo pine (*Pinus halepensis*) plantations, the upper part of the plateau is mainly covered by semi-natural dry grasslands. To date, grasslands cover approximately 29,800 ha in Alta Murgia and represent the remnants of the approximately 80,000 ha wide grasslands which existed at the beginning of the 20th century. Calcareous grasslands of the area were formed by various natural and anthropogenic processes and are mainly managed by moderate sheep grazing (MAIROTA et al. 2013). Among the grassland plant communities occurring in this area, those belonging to the phytosociological classes *Festuco-Brometea* Br.-Bl. et Tx. ex Soó 1947, *Stipo-Trachynietea distachyae* S. Brullo in S. Brullo et al. 2001 and *Lygeo sparti-Stipetea tenacissimae* Rivas-Mart. 1978 are listed in the Habitats Directive 92/43/EEC (6220*: Pseudo-steppe with grasses and annuals; and 62A0: Eastern sub-Mediterranean dry grasslands). Due to small-scale heterogeneity in soil morphology and past land use, these grassland communities are commonly found as complex mosaics or transitional forms in many sites of Alta Murgia (LABADESSA et al. 2017). Only a few studies have considered successional dynamics and restoration potential of these important grassland types.

The study area is a restored landfill abandoned since 1992 and restored in 2015. After removing waste, the landfill was capped using different layers (clay, HDPE geomembrane, drainage geonet), then covered with native fine-grained soil for vegetation recovery. Our
study focused on the central flat part of the area, where a uniform soil layer (> 30 cm thick) was laid. A portion of the restored area (1.46 ha) was evenly grazed by 4 sheep/hectare, which were fenced in the area in the morning during spring and autumn. The remaining portion (0.36 ha) was not grazed as it was protected by an inner fence (Fig. 1). Both grazed and ungrazed portions of the restored landfill were surrounded by continuous semi-natural grasslands, representing a reference for vegetation recovery. The grassland vegetation was composed by a mosaic of annual and perennial plant communities developing on rocky calcareous soil and is mostly managed by extensive sheep grazing. The grasses *Dasylyrum villosum*, *Dactylis glomerata* subsp. *hispanica* and *Triticum ovatum* were the most abundant species in the area and represented over 50% of total vegetation cover in the grasslands. Among forbs, *Hypochaeris achyrophorus*, *Thapsia garganica* and *Trifolium stellatum* were typical, with mean cover values above 3%.

3. Methods

Vegetation data were collected in 23 permanent plots (1 m × 1 m), marked with PVC sticks, in the restored area during the first three years after landfill restoration, i.e. in May of 2016, 2017 and 2018. Plot size was chosen because this is among the most frequently used plot sizes in grasslands (please see GrassPlot 2.0, the most comprehensive database on Palaearctic grasslands, Biurrun et al. 2019). Plots were evenly distributed in the area, at reciprocal distance of more than 10 meters. Given the different size of the grazed and ungrazed areas, we considered 17 grazed plots and 6 ungrazed plots. Although the study area had a limited width, it comprised different small-scale herbaceous communities.

We recorded the presence and percentage cover of all vascular plant species, total vegetation cover and mean vegetation height in each sampling plot across three years. In order to assess a reference for vegetation recovery, vegetation data were collected during May 2018 in 10 plots (1 m × 1 m) in the surrounding grassland (2.67 ha), in a distance of 2m-30 m from the edge of the restored area. Plant nomenclature follows Bartolucci et al. (2018).

To assess the species composition of the 23 plots in the three years, a DCA ordination based on percentage cover of the species was calculated using CANOCO 5.0 program (Ter Braak & Šmilauer 2012). A set of plant community descriptors referring to structure (vegetation mean height, total vegetation cover) and composition (species richness, Shannon diversity) was then used for the subsequent analysis.

Species were classified according to their morphology (i.e. grasses or forbs) and their function (i.e. target species or weeds in the reference vegetation). Target species were classified based on their phytosociological affiliations to the classes *Festuco-Brometea*, *Stipo-Trachynietea distachyae* and *Lygeo sparti-Stipetea tenacissimae* (Mucina et al. 2016). Weeds were identified as ruderal species or remnants of earlier successional stages, which typically do not occur in the reference grasslands.

All species were also classified according to their dispersal potential through epizoochory. For this we assigned an epizoochory ranking index (ERI) for every species, provided by the D3 database (Hintze et al. 2013). ERI values for species not reported in the D3 database were calculated as average ERI values of closely related species of the same genus or family. Species were then classified as “good dispersers” and “poor dispersers”, respectively considering species with values above or below the median of ERI of the sampled flora (ERI = 0.6).

Relative Response Indices (RRI; Brinkman et al. 2010) were calculated for describing the relationships between the vegetation characteristics (i.e. species richness, Shannon diversity, total vegetation cover, average vegetation height and the cover scores of the functional groups) of the restored area and the reference grassland. RRI has been recently used for the assessment of plant community succession in restoration studies (Valkó et al. 2017), and was calculated using the following equation:

\[
RRI = (C_R - C_d)/(C_R + C_d)
\]
where \( C_R \) represents the vegetation characteristic (i.e. percentage cover of a particular functional group) of the restored area and \( C_G \) represents the vegetation characteristic of the reference grassland. Value of RRI ranges from -1 to +1. The closer is the RRI to zero, the higher the similarity of the recovering vegetation to the reference grassland, while the closer |RRI| to 1, the lower the similarity. Negative RRIs mean that a certain variable has a smaller value in the restored area, positive RRIs mean that the variable has a larger value in the restored area compared to the reference grassland. To analyse restoration success, RRIs of the vegetation characteristics in the three study years were tested by repeated measures general linear models accounting for the normal distribution of the dependent variables. We used management (two levels: grazed, ungrazed) and year (three levels: Year 1, Year 2 and Year 3) as fixed factors. The values of the dependent variables were log-transformed (using the \( \log(x+2) \) formula) to approximate them to normal distribution. All univariate statistics were calculated using the GLM repeated measures command in IBM SPSS Statistics v. 20.0 (Amonk, NY: IBM Corp).

4. Results

4.1 Species composition of the restored area

Among the 189 vascular plant species recorded in the study, 21, 31 and 38 species were respectively recorded in the restored area in the first, second and third year after restoration, with mean species richness (± SD) of 7.13 ± 2.67 per sample plot. The reference grassland harboured a total of 142 species, with mean species richness of 31.02 ± 12.23 per sample plot. According to the DCA ordination, plant community composition shifted in the restored area with successional age and grazing regime (Fig. 3). The primary component revealed a gradient of increasing similarity to the reference grassland, with species characteristic for the reference grassland plotted towards the third year plots. The ordination segregated grazed communities from ungrazed ones along the second axis. Plant assemblages dominated by ruderal species (e.g., \( Avena barbata \), \( Conium maculatum \) and \( Silybum marianum \)) were associated with first year samples and ungrazed plots, while species assemblages in the following years became gradually richer in target grassland species (\( Bartsia trixago \), \( Trifolium campestre \) and \( Trifolium scabrum \)), especially in grazed plots (Fig. 3).

4.2 Relative response indices

For the majority of considered vegetation characteristics, scores in the restored area became more similar to the reference grassland (i.e. RRIs were closer to zero), both along successional age and grazing (Fig. 4, Supplement E1). The 1-year-old restored grassland was characterised by significantly lower total vegetation cover and higher vegetation height than the reference grassland (Table 1). In the restored grassland, vegetation height became more similar to the reference grassland with increasing successional age regardless of management type (Fig. 4, Table 1). Total vegetation cover at the restored area became more similar to that of the reference grassland with increasing successional age and grazing (Fig. 4, Table 1). The considered factors also had a positive effect on species richness (Fig. 4) and successional age had a negative effect on Shannon diversity (Fig. 4). First-year plots were all different from the reference grassland in terms of target and weed species cover. Successional age and grazing, both considered individually and coupled, had positive effects on the RRIs of target forb cover (Fig. 4). The cover of weedy forbs became significantly more similar to the reference grassland with successional age (Fig. 4, Table 1). The cover of target grass species was not significantly affected either by increasing successional age or grazing (Fig. 4, Table 1).
However, the RRI of the cover of non-target grass *Dasypyrum villosum*, which was the most abundant in the reference grassland (average cover 20%), showed a rapid increase in the restored site (RRI_{Year 1}: -0.77 ± 0.29; RRI_{Year 2}: -0.10 ± 0.65; RRI_{Year 3}: -0.06 ± 0.60). The interaction of age and grazing had a significant effect on the RRIs of the cover of weedy grasses (Table 1). The cover of good epizoochorous dispersers was higher during later stages of succession and became more similar to that of the reference grassland in case of grazing (Fig. 4, Table 1). Grazing also had negative effect on the cover of poor dispersers, while the coupled effect of increasing successional age and grazing affected the cover of both good and poor dispersers (Fig. 4, Table 1).

## 5. Discussion

In this study we analysed changes in plant communities on newly created open soil surfaces in order to explore the role of sheep grazing in promoting spontaneous vegetation recovery, with reference to adjacent dry grassland vegetation. Our study revealed that if restoration sites are surrounded by natural grasslands, spontaneous plant recovery can provide promising results even within a few years. Cover of target grassland forbs in
the restored area approached that present in reference grassland during the three years of the study, indicating that many target species can quickly establish in the restoration area from the surrounding areas via seed rain. This is in line with studies demonstrating that adjacent grasslands represent an important source habitat from which species colonize after restoration (BAKKER & BERENDSE 1999, ÖSTER et al. 2009, WINSA et al. 2015). Thus, this method should be first considered for sites where there is a high chance for unassisted establishment of target species (WALKER et al. 2014). Passive restoration is more cost effective compared to species introduction measures (e.g. by seed sowing), can support sustainable regeneration pathways (PRACH & REHOUNKOVA 2008, ALBERT et al. 2014) and can facilitate species and functional diversity (TÖLGYESI et al. 2019). As target species are able to establish from the surroundings, this may also ensure colonization by locally adapted ecotypes.

This was not true for target grasses, which did not approach the reference grassland in terms of cover and species composition. A possible reason is that during spontaneous recovery, target grasses usually colonize the restored sites during mid-successional stages (see e.g. ALBERT et al. 2014, NOVÁK & PRACH 2003). Another possible reason is that in our study, the non-target grass Dasypyrum villosum showed a rapid increase in the restored site and probably hampered the establishment of other grass species. These results suggest that even though sheep grazing was a good tool for supporting grassland recovery and several important target species of the reference grasslands established already in the first three years, the spontaneous establishment of target grasses will require more time.

The faster recovery in grazed restored grassland also suggests that extensive grazing management can be a feasible tool for facilitating spontaneous recovery of dry grasslands. Given that proximity to propagule sources and lack of proper dispersal vectors is often limiting grassland recovery (OZINGA et al. 2009, DEÁK et al. 2015), the presence of livestock grazing can be a very effective measure for increasing species richness through zoochorous seed dispersal (FISCHER et al. 1996, ROSENTHAL et al. 2012, WINSA et al. 2015, TÖTH et al. 2018). Gaps created by extensive trampling can provide microsites for the establishment of subordinate target species (DÍAZ et al. 2001, TARHOUNI et al. 2015, EICHBERG & DONATH 2018). In particular, we found that both target species and plant species best adapted to

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Fig. 4. Relative Response Indices (RRIs) of vegetation height, total vegetation cover, target forb and grass cover, weedy forb and grass cover, and cover of good and poor dispersers (mean ± SE). RRIs were calculated in the restored area at Year 1, Year 2 and Year 3. Full symbols denote scores calculated for the grazed plots, while empty symbols denote ungrazed plots. The results of the repeated measures linear models for Age, Management and Age × Management (A × M) are displayed for all dependent variables; notations: *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$, n.s. – not significant. See Supplement E1 for corresponding statistical analyses.

Abb. 4. Relative Response Indices (RRIs) für die mittlere Vegetationshöhe, Gesamtdeckung der Vegetation, Deckung der krautigen und grasartigen Zielarten, und Deckungsgrad der Arten mit gutem und schwachem epizoochoren Ausbreitungsvermögen (Mittelwert ± Standardabweichung). RRIs wurden in den renaturierten Flächen im 1., 2. und 3. Jahr berechnet. Die gefüllten Symbole zeigen Werte für die beweideten Flächen an, während die ungefüllten Symbole Werte für die unbeweideten Flächen darstellen. Die Ergebnisse der allgemeinen linearen Modelle mit wiederholten Messungen für die Faktoren Alter, Management und Alter × Management (A × M) sind für alle abhängigen Variablen angezeigt; Abkürzungen: *** $p \leq 0.001$; ** $p \leq 0.01$; * $p \leq 0.05$; n.s. – nicht signifikant. Siehe Anhang E1 für die dazugehörige statistische Analyse.
Table 1. Parameter values (mean ± SD) recorded in grazed and ungrazed sites during the three years, compared with reference grassland.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Grazed sites</th>
<th>Ungrazed sites</th>
<th>Reference grassland</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Year 1</td>
<td>Year 2</td>
<td>Year 3</td>
</tr>
<tr>
<td>Total cover</td>
<td>58.24 ± 8.56</td>
<td>88.24 ± 12</td>
<td>93.53 ± 7.62</td>
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<tr>
<td>Species richness</td>
<td>6.53 ± 1.68</td>
<td>7.47 ± 2</td>
<td>9 ± 3.6</td>
</tr>
<tr>
<td>Shannon diversity</td>
<td>1.48 ± 0.22</td>
<td>1.28 ± 0.41</td>
<td>1.28 ± 0.42</td>
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<tr>
<td>Target forb cover</td>
<td>3.35 ± 2.83</td>
<td>15.65 ± 14.58</td>
<td>29.35 ± 22.59</td>
</tr>
<tr>
<td>Target grass cover</td>
<td>0 ± 0</td>
<td>0.35 ± 1.19</td>
<td>2.59 ± 8.23</td>
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<tr>
<td>Weedy forb cover</td>
<td>21.24 ± 7.21</td>
<td>5.41 ± 5.71</td>
<td>2.59 ± 3.74</td>
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<tr>
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<td>16.47 ± 14.93</td>
<td>11 ± 16.44</td>
<td>5.71 ± 15.12</td>
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<tr>
<td>Good dispersers cover</td>
<td>23.59 ± 10.38</td>
<td>65.47 ± 25.06</td>
<td>66.82 ± 23.9</td>
</tr>
<tr>
<td>Poor dispersers cover</td>
<td>37.53 ± 16.44</td>
<td>22.41 ± 20.01</td>
<td>26 ± 21.47</td>
</tr>
</tbody>
</table>

Reference grassland
Epizoochory became more abundant in the grazed area, which underlined the role of sheep as an important driver of seed dispersal of grassland species (Dostálek & Frantík 2008, Freund et al. 2014, Klemens et al. 2014). Sheep are particularly useful for restoration as they tend to be kept in large herds which are relatively easily transferable and possess fur that has a high seed transport capability (Wessels et al. 2008).

Site managers are often concerned about the applicability of grazing in early-successional restoration sites, because animal trampling can create suitable establishment gaps for weedy species (Valkó et al. 2016, Eichberg & Donath 2018). In some cases, grazing can undermine biodiversity benefits (Lindenmayer et al. 2018). However, in our study system, grazing in the early successional years did not increase the cover of weeds and instead proved to be effective in promoting species diversity and cover of target grassland species. This implies that site managers can minimise the costs of restoration as there is no need for installing protective fences around restored areas. It is very important from the viewpoint of sustainability, that restored sites can provide forage for the livestock and support local animal husbandry. In the study region, as well as in other countries of the European Union, this means that site managers could be eligible for agri-environmental subsidies, which could increase the feasibility and sustainability of restoration projects. An involvement of local farmers from the very beginning could increase their commitment throughout the project.

**Erweiterte deutsche Zusammenfassung**


Acknowledgements

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Author contribution

RL, BD and OV conceived and designed the research; RL collected the data; RL, BD and OV analyzed the data, wrote and edited the manuscript. All authors gave final approval for publication.
Supplements

Additional supporting information may be found in the online version of this article.

**Supplement E1.** Effects of successional age, grazing management and coupled effect of age × management on Relative Response Index [RRI] scores of the vegetation characteristics as tested by repeated-measures general linear models.


**References**


Supplement E1. Effects of successional age (repeated measures factor), grazing management (fixed factor) and coupled effect of age × management on Relative Response Index (RRI) scores of the vegetation characteristics (i.e. scores of the restored area compared to that of the reference grassland) as tested by repeated-measures general linear models. F-values for age and age × management were derived from the within-subject analysis part, and the F-value for management from the between-subjects analysis part. For calculating the degrees of freedoms in the within subject analysis we used Greenhouse-Geisser correction.


<table>
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<th>Parameter</th>
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<td>1.132</td>
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<td>2.164</td>
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<td>1.129</td>
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<td>1.591</td>
<td>86.824</td>
<td>0.000</td>
<td></td>
<td>1</td>
<td>9.426</td>
<td>0.006</td>
<td></td>
<td>1</td>
<td>1.591</td>
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<td>1.344</td>
<td>10.082</td>
<td>0.005</td>
<td></td>
<td>1</td>
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<td>9.286</td>
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<td>3.804</td>
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<td>1.007</td>
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<tr>
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<td>1.792</td>
<td>9.934</td>
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<td>0.153</td>
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