Up in the Tree – The Overlooked Richness of Bryophytes and Lichens in Tree Crowns

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Abstract

Assessing diversity is among the major tasks in ecology and conservation science. In ecological and conservation studies, epiphytic cryptogams are usually sampled up to accessible heights in forests. Thus, their diversity, especially of canopy specialists, likely is underestimated. If the proportion of those species differs among forest types, plot-based diversity assessments are biased and may result in misleading conservation recommendations. We sampled bryophytes and lichens in 30 forest plots of 20 m × 20 m in three German regions, considering all substrates, and including epiphytic litter fall. First, the sampling of epiphytic species was restricted to the lower 2 m of trees and shrubs. Then, on one representative tree per plot, we additionally recorded epiphytic species in the crown, using tree climbing techniques. Per tree, on average 54% of lichen and 20% of bryophyte species were overlooked if the crown was not included. After sampling all substrates per plot, including the bark of all shrubs and trees, still 38% of the lichen and 4% of the bryophyte species were overlooked if the tree crown of the sampled tree was not included. The number of overlooked lichen species varied strongly among regions. Furthermore, the number of overlooked bryophyte and lichen species per plot was higher in European beech than in coniferous stands and increased with increasing diameter at breast height of the sampled tree. Thus, our results indicate a bias of comparative studies which might have led to misleading conservation recommendations of plot-based diversity assessments.

Introduction

Assessing diversity is a major task in ecology and conservation science. Understanding patterns of diversity and guiding conservation decision-making both rely on an unbiased assessment of diversity, for instance of species richness per given area and given sampling effort [1,2]. In plants, diversity assessments are usually plot-based and all species up to accessible heights are sampled (e.g. [3,4]). However, this approach may lead to underestimating the diversity of species not occurring on the ground (i.e. epiphytes). Furthermore, if the proportion of those underestimated species differs among habitat types or varies systematically with some environmental drivers, plot-based diversity assessments will be biased and may result in misleading conservation recommendations.

Bryophytes and lichens are two very diverse groups of cryptogams, which occur on a wide range of substrates, inhabit essentially all terrestrial and some aquatic habitats and many of them live as epiphytes [5,6]. In forests, the species richness and composition of cryptogam communities varies among geographical regions, forest management types, main tree species, and stand ages [7-10]. On individual trees, the species composition of epiphytic bryophyte and lichen communities changes with tree age, because of changing bark structure as well as quality, and with tree height as abiotic and biotic conditions change from trunk bases to the uppermost twigs [11-15].

Ecological studies, which monitored bryophytes and lichens or compared their species richness in forests among different regions, management types or tree species composition usually sampled all species of a defined area [7,8,15] or only epiphytes on trunks of individual trees [16-18]. However, in these surveys epiphytic species were only sampled up to accessible heights (e.g. 2 m above ground). Thus, epiphytic bryophytes and lichens in tree crowns are rarely investigated, especially for forests in temperate Europe. All previous studies describing the vertical distribution, composition, richness or biomass of epiphytic bryophytes and lichens have been...
How many species per tree and per plot are on average overlooked without sampling bryophytes and lichens in tree crowns? conducted outside continental Europe and mainly investigated single or few fallen or cut trees (e.g. [15,19-35]). Only few studies quantified the number of epiphytic bryophyte and lichen species, which are exclusively growing in tree crowns and compared this number to the one of the trunk of the same tree below 2 m [30,35]. However, none of these studies compared the species richness of epiphytic bryophytes and lichens in tree crowns with the total species richness of ground plots, which would have allowed them to assess the number and proportion of overlooked species during regular surveys. Some epiphytes may be recorded on the ground because of epiphytic litter fall and of species growing on fallen branches and twigs, but the true number and proportion of crown specialists remains unknown without surveying tree crowns [36]. Thus, it also remains unclear how many epiphytic bryophyte and lichen species are on average overlooked in regular cryptogam surveys. Furthermore, whether the number or proportion of these overlooked species is consistent among geographical regions, forests types, or stand ages has never been comprehensively analyzed in a comparative study.

Therefore, we sampled the species richness of bryophytes and lichens in European beech (Fagus sylvatica L.) and coniferous forests of different stand ages, in three German regions, distinguishing species recorded in a regular survey of all substrates on a plot, epiphytic species occurring only at the base of trees, and epiphytic species occurring only in the crown of trees.

Our main questions were:

1. How many species per tree and per plot are on average overlooked without sampling bryophytes and lichens in tree crowns?

2. Does the number and proportion of overlooked species vary among regions, tree species, and tree age?

Materials and Methods

Study system

This study was conducted as part of the Biodiversity Exploratories project ([37]; www.biodiversity-exploratories.de) in three regions of Germany: (1) the UNESCO Biosphere area Schwäbische Alb (Swabian Jura), situated in the low mountain ranges of South-western Germany, (2) the National Park Hainich and its surrounding areas, situated in the hilly lands of Central Germany, and (3) the UNESCO Biosphere Reserve Schorfheide-Chorin, situated in the young glacial lowlands of North-eastern Germany. The three study regions each extend over at least 20 by 30 km. They differ in climate, geology, and topography from each other, while harbouring forest types typical for large parts of temperate Europe (Table 1). AMONG the forest plots of the Biodiversity Exploratories, we selected the subset of 30 plots (9 plots each in the Schwäbische Alb and Schorfheide-Chorin, and 12 plots in the Hainich-Dün region) dedicated to intensive research. In each region, three plots contain coniferous stands dominated by Norway spruce (Picea abies L.) H.Karst.) in the Schwäbische Alb and Hainich-Dün, and Scots pine (Pinus sylvestris L.) in the Schorfheide-Chorin, and six to nine stands dominated by European beech [37].

Field work permits were issued by the responsible state environmental offices of Baden-Württemberg, Thüringen, and Brandenburg (according to § 72 BbgNatSchG).

Table 1. Characteristics of the three Biodiversity Exploratories.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Schwäbische Alb</th>
<th>Hainich-Dün</th>
<th>Schorfheide-Chorin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location</td>
<td>SW Germany</td>
<td>Central Germany</td>
<td>NE Germany</td>
</tr>
<tr>
<td>Size (km²)</td>
<td>~422</td>
<td>~1300</td>
<td>~1300</td>
</tr>
<tr>
<td>Geology</td>
<td>Calcareous bedrock</td>
<td>Calcareous bedrock</td>
<td>Young glacial landscape</td>
</tr>
<tr>
<td>Altitude a.s.l. (m)</td>
<td>460–860</td>
<td>285–550</td>
<td>3–140</td>
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<tr>
<td>Mean annual temperature (°C)</td>
<td>6.0–7.0</td>
<td>6.5–8.0</td>
<td>8.0–8.5</td>
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<tr>
<td>Mean annual precipitation (mm)</td>
<td>700–1000</td>
<td>500–800</td>
<td>500–600</td>
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<tr>
<td>Main tree species</td>
<td>European beech</td>
<td>European beech</td>
<td>European beech</td>
</tr>
<tr>
<td></td>
<td>Norway spruce</td>
<td>Norwegian spruce</td>
<td>Scots pine</td>
</tr>
<tr>
<td>Number of plots</td>
<td>6</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>Mean (SD) DBH (m)</td>
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<td>0.30 (0.13)</td>
<td>0.58 (0.21)</td>
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<td>0.19–0.45</td>
<td>0.32–0.86</td>
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<td>Deadwood volume (m³)</td>
<td>0.028 (0.019)</td>
<td>0.022 (0.016)</td>
<td>0.028 (0.016)</td>
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<tr>
<td>Range</td>
<td>0.007–0.064</td>
<td>0.012–0.040</td>
<td>0.010–0.051</td>
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<td>Ground cover by rocks (%)</td>
<td>0.7 (0.9)</td>
<td>0.0 (0.0)</td>
<td>0.2 (0.4)</td>
</tr>
<tr>
<td>Range</td>
<td>0.0–2.5</td>
<td>–</td>
<td>0.0–1.0</td>
</tr>
<tr>
<td>Ground cover by bryophytes (%)</td>
<td>1.2 (0.7)</td>
<td>56.0 (46.2)</td>
<td>3.1 (3.0)</td>
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<tr>
<td>Range</td>
<td>0.5–2.0</td>
<td>5.0–95.0</td>
<td>0.5–3.0</td>
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Table 2. GLM results for differences in the number of overlooked bryophyte and lichen species among the three regions, European beech vs.

<table>
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<tr>
<th>Source of variation</th>
<th>Overlooked species per tree</th>
<th>Overlooked species per plot</th>
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<tr>
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<td>Lichens</td>
<td>Bryophytes</td>
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<td>Region</td>
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<tr>
<td>DBH</td>
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<td>Interactions</td>
<td></td>
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<td>Beech vs. conifer × DBH</td>
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<td>0.78</td>
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<tr>
<td>Region × beech vs. conifer</td>
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<td>7.47</td>
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<tr>
<td>Region × DBH</td>
<td>2</td>
<td>2.40</td>
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<tr>
<td>Residual Deviance</td>
<td>20</td>
<td>66.54</td>
</tr>
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</table>

Significant differences are indicated by bold p values. doi: 10.1371/journal.pone.0084913.t002

Bryophyte and lichen sampling

Nomenclature of lichens follows Wirth et al. [38], the one of bryophytes Nebel & Philippi [39-41]. In spring 2008, we first identified bryophyte and lichen species on all 30 plots of 20 m × 20 m, separated by substrate type (bark, rocks, deadwood, and soil), including epiphytic species which were growing on fallen twigs and branches from the tree crown. The sampling of species on bark was restricted to the lower 2 m of trees and shrubs. From this data, we calculated the total number of bryophyte and lichen species occurring in the lower 2 m of the plot. We then selected one tree per plot, which was representative for other trees occurring in the particular plot in terms of tree species identity, height and diameter at breast height (DBH), and recorded epiphytic bryophyte and lichen species which grew in the crown. We used single and double rope climbing techniques, which allowed us to sample approx. 70% of the whole tree crowns, except the outermost branches. We did not limit our sampling time and searched until no more new species were found. We further distinguished species by their substrate affinity into obligate epiphytes, facultative epiphytes, and non-epiphytes according to Wirth et al. [38] for lichens and Nebel & Philippi [39-41] for bryophytes (see Table S1) excluding the ones only identified to genus identity. Differing from Wirth et al. [38] we categorized the lichen Trapeliopsis flexuosa as facultative epiphyte instead of a non-epiphyte because the species also occurred as epiphyte in one of our plots. For the same reason we did that also for the bryophyte species Atrichum undulatum, Eurhynchium praelongum, Plagiomnium undulatum, Polytrichum formosum, Rhytididaphus loreus, and Thuidium tamariscinum which were listed as non-epiphytes in Nebel & Philippi [39-41].

From this data we calculated the number of species per tree, dividing them into those exclusively growing on the trunk below 2 m, exclusively growing in the crown, and shared species between the trunk and the crown. However, as Orthotrichum stramineum was according to Nebel & Philippi [39-41] the only obligate epiphytic bryophyte species in our data set, we did not analyze the three bryophyte groups separately.

Plot characteristics

We measured the DBH of the investigated trees, which has been shown to be related to tree age [42] and the surface area of the tree crown [43]. Furthermore, we estimated the ground cover by bryophytes and rocks, and measured length and diameter of all deadwood items (≥7 cm of diameter) to estimate deadwood volume assuming cylinder shapes (see Table 1).

Statistical analysis

We analyzed the number of overlooked bryophyte and lichen species per tree and per plot using GLM models with Poisson errors and corrected for overdispersion, when necessary. Explanatory variables were the three study regions, tree species identity (European beech vs. conifer), and DBH of the sampled tree. Furthermore, we included interactions between explanatory variables. F- and Chi-square tests were used to test the significance of deviance changes associated with factors added sequentially to the model (sequence shown in Table 2). Model simplification did not change the results and we therefore present the full models. Data were analyzed using R, Version 2.13.1 [44].

Results

Overall and regional species richness

Over all 30 plots, we recorded 99 lichen and 88 bryophyte species. Of the 99 lichen species, 80 were epiphytes (38 obligate epiphytes according to Wirth et al. [38]), 11 were growing on deadwood, 15 on rocks, and 21 on fallen twigs and branches from the tree crown. Interestingly, we found no lichen species growing on soil. Of the 88 bryophyte species, 51 were epiphytes (1 obligate epiphyte according to Nebel & Philippi [39-41]), 38 were growing on deadwood, 29 on rocks, 38 on soil, and 5 on fallen twigs and branches from the tree crown. In the Schwäbische Alb region we recorded 87 lichen and 69 bryophyte species, in Hainich-Dün 25 and 46, and in Schorfheide-Chorin 38 and 30, respectively. Fifty-six lichen and 34 bryophyte species were recorded exclusively in the Schwäbische Alb region, 1 and 6 in Hainich-Dün, as well as 11
and 6 in Schorfheide-Chorin, respectively. Over all three regions, we recorded 15 epiphytic lichen and 3 epiphytic moss species only in tree crowns (for details see Table S1).

The number and proportion of overlooked epiphytic lichen and bryophytes species

At the level of a single tree, a substantial number of epiphytic lichen and bryophyte species were overlooked if the crown had not been sampled. On average, we recorded 11.2 (± 10.3, mean ± standard deviation) epiphytic lichen species per tree of which 6.0 (± 6.4) species occurred exclusively in the crown. For bryophytes, we recorded an average of 6.0 (± 3.8) epiphytic species per tree, of which 1.2 (± 1.5) occurred exclusively in the crown. These data indicate that on average 54% of lichens and 20% of bryophytes were overlooked per tree when the crown was not sampled (Figure 1A, 2A).

At the level of the plots, by also sampling the crown of one tree per plot we recorded 38% more lichen species and 4%
more bryophyte species (Figure 1B, 2B) than by sampling the bark below 2 m and the ground substrates only. Comparing these values with individual tree data indicates that other substrates in a plot harbour a large part of the bryophyte species typical for tree crowns, whereas this was not the case for lichens. Classifying bryophyte and lichen species by their substrate affinity into obligate epiphytes, facultative epiphytes, and non-epiphytes did not qualitatively change the results (Figure S1). These findings indicate that the number of overlooked species per plot is hardly driven by obligate epiphytic species.

Differences in the number and proportion of overlooked epiphytic lichen and bryophyte species among regions and forest stands

For lichens, the number and proportion of overlooked epiphytic species varied strongly among regions: At the level of trees, on average in the Schwäbische Alb 12.1 (± 2.7, mean ± standard error; 58% of all species), in Hainich-Dün 3.3 (± 1.0; 52%), and in Schorfheide-Chorin 3.4 (± 0.9; 43%) epiphytic lichen species were overlooked if the crown had not been sampled (Figure 1A). At the level of plots, on average in the Schwäbische Alb 8.3 (± 2.5; 26%), in Hainich-Dün 2.7 (± 0.9; 39%), and in Schorfheide-Chorin 2.6 (± 0.7; 22%) epiphytic lichen species were overlooked if the crown of one tree had not been sampled (Figure 1B). Furthermore, on average the number of overlooked epiphytic lichen species per plot was higher in European beech (5.1 ± 1.2 species; 28%) than in coniferous stands (2.4 ± 1.3 species; 26%; Figure 1B; Table 2).

Finally, the number of overlooked epiphytic lichen species increased by approximately 1 species with an increase of 0.1 m in DBH of the sampled tree, both at the level of trees and plots. These findings indicate that the extent to which species richness was underestimated by ignoring tree crowns varied as a function of local conditions and stand characteristics, and thereby affects biodiversity assessments differently.

For bryophytes the number and proportion of overlooked epiphytic species per tree and per plot did not vary among regions. On average, the number of overlooked epiphytic bryophyte species per tree was higher on European beech (1.7 ± 0.3 species; 24%) than on coniferous trees (0.1 ± 0.1 species; 3%) if the crown had not been sampled (Figure 2A). Furthermore, on average the number of overlooked epiphytic bryophyte species per plot was higher in European beech (0.8 ± 0.2 species; 5%) than in coniferous stands (0.0 ± 0.0 species; 0%) if the crown of one tree had not been sampled (Figure 2B, Table 2). The number of overlooked epiphytic bryophyte species increased by 0.13 species per plot with an increase of 0.1 m in DBH of the sampled tree. These results indicate that bryophyte diversity is underestimated by ignoring tree crowns, especially in beech forests.

Discussion

Overlooked epiphytic bryophyte and lichen species per tree and plot

We found high numbers of species exclusively growing in the canopy of the sampled tree, which are overlooked when only the first 2 meters of the tree trunk are sampled. This number still remained high, at least for lichen species, when all other substrates as well as epiphyte litter fall in a plot were sampled. At the level of trees, lichen [30,35] or bryophyte species [30] exclusive to the crown have rarely been addressed for European forests and not at all for temperate European ones. Among the few studies, Marmor et al. [35] recorded lichens on 15 Norwegian spruces and 15 Scots pines in southern Estonia and concluded that approximately two third of the trees’ lichen species are growing exclusively in the canopy. Fritz [30] sampled species of conservation concern on European beeches of three age classes in Sweden and found both bryophyte and lichen species which were restricted to the crown of trees. However, across all investigated trees, he found all but one lichen species of conservation concern growing also below 2 m and concluded that the regular surveying method is applicable and efficient for a high number of stems if the aim of a study is to record species of conservation concern within a stand.

However, none of the previous studies related their findings of differences in bryophyte and lichen species between trunks and crowns of individual trees to the level of a plot with a defined area, although this is the most common method in vegetation surveys with the aim to compare the species richness among different forest types. Thus, it has not been tested how many species on average are overlooked during regular plot-based surveys. We showed that, even after sampling the lower bark of all other trees of the plot, as suggested by Fritz [30] to reduce the number of underestimated species, and all other substrates and epiphyte litter fall, the number of overlooked epiphytic species still remained high, at least for lichen species. For bryophytes, in contrast, sampling of epiphyte litter fall and other substrates seems to reduce the number of overlooked species per plot to a negligible overall value.

Variation in overlooked epiphytic bryophyte and lichen species among regions and forest stands

In a larger study from the same regions which were investigated here, Boch et al. [8] sampled 631 forest plots with regular sampling methods and reported regional differences in lichen species richness, which were most likely related to the former intensity of atmospheric pollutants. In the current study, the number of overlooked lichen species per plot also differed among regions, whereas this was not the case for bryophytes. This is clearly calling for caution when attempting to generalize results among regions and taxa for proposing conservation recommendations.

With regard to the overall bryophyte species richness on a plot up to 2 m height, Humphrey et al. [45], who compared deciduous stands and conifer plantations in Britain and found almost exclusively species growing on deadwood, observed no differences in bryophyte richness between deciduous stands and conifer plantations. This is in contrast to our findings of higher bryophyte species richness in coniferous than European beech stands, which was mainly because of generally higher numbers of bryophyte species growing on soils of coniferous stands. In coniferous stands, soil-dwelling bryophytes profit...
from higher light availability on the forest floor due to less closed canopy cover [46] and reduced litter fall compared with generally unfavorable conditions in European beech forests [47]. However, for lichens we found the opposite of higher overall numbers in European beech than in conifer stands because of higher numbers of epiphytic species. Similarly, Marmor et al. [35] and Humphrey et al. [45] reported differences in the total number and composition of lichen species among tree species. One explanation might be that native tree species provide a more suitable habitat for epiphytic species than non-native ones. In our case, European beech or mixed beech forests would most likely form the potential natural vegetation of all our investigated plots and all coniferous stands are plantations [48]. Furthermore, all investigated coniferous plantations resulted from clear cuts which are poor in species depending on stand continuity [8].

With regard to the overlooked epiphytic bryophyte and lichen species on the level of a plot, their number was generally lower in coniferous than European beech stands. This pattern was more pronounced for lichen than for bryophyte species, reflecting generally high numbers of crown specific lichen species. In contrast, crown-specific bryophyte species were almost completely lacking on coniferous trees. One explanation could be that the number of microhabitats for specialized bryophytes, such as rot holes and pronounced bark textures [49] is lower in the crowns of coniferous trees than of European beeches. In addition, microclimatic variables such as light and humidity conditions might likely differ among tree species. However, coniferous trees generally appear to provide less favorable conditions for epiphytic bryophytes than deciduous ones (e.g. [11,50,51]).

In general, the number of overlooked bryophytes and lichens per tree and plot increased with increasing tree age, indicated by the DBH of the investigated tree. This is most likely because older trees provide a range of microhabitats, which make them better suitable for more specialized bryophyte and lichen species (e.g. [30,49,52,53]).

In conclusion, the number of overlooked bryophyte and lichen species differs among regions and forest types, indicating a bias of comparative studies which might have led to misleading conservation recommendations of plot-based diversity assessments. Even if this bias was smaller for bryophytes than for lichens, with relatively low numbers of overlooked species per plot, it was still significant.

**Recommendations for diversity assessments**

Our study emphasizes the importance of tree crown sampling for complete plot-based diversity assessments. As the number of tree crown specialists differs among regions and forest types, it appears indispensable to correct for such differences before comparing their species richness. Thus, further studies are needed developing specific correction factors, which would allow an objective comparison of lichen and bryophyte species richness among forest types and regions, leading to unbiased conservation recommendations. Up to now it remains unclear, how many tree crowns per region, forest type, and plot have to be investigated to capture all canopy specialists and calculate a representative correction factor. Furthermore, it is open how increasing tree diversity is affecting the sampling bias. We are aware that time-consuming methods, such as tree climbing, are not always applicable in regular surveys, in particular, when high numbers of plots need to be sampled. Instead of climbing, bryophytes and lichens can also be sampled from the crowns of recently fallen or cut trees, which represent the majority of the stand dominating trees in terms of tree species identity and tree age.

**Supporting Information**

**Figure S1.** Mean number of lichen species (+SE) separated by substrate affinity (according to Wirth et al. [38]) growing A) exclusively on the trunk below 2 m, exclusively in the crown, or in both parts of the sampled tree, and B) exclusively on the plot below 2 m, exclusively in the crown of the sampled tree, or in both parts of the plot, separated for the main tree species per plot for all three study regions. Sample size is indicated by the numbers above the bars.

**Table S1.** Presence of lichen and bryophyte species separated by substrate affinity (according to Wirth et al. [38] for lichens and Nebel & Philippi [39-41] for bryophytes) and the substrate on which they occurred: i) in the crown and ii) on the trunk of the sampled tree, and iii) on other substrates in the plot excluding the sampled tree. Species marked with an asterisk were only found in tree crowns.

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**Author Contributions**

Conceived and designed the experiments: S. Boch DP MF. Performed the experiments: S. Boch JM S. Blaser. Analyzed the data: S. Boch DP. Wrote the manuscript: S. Boch. Provided editorial advice: JM DP S. Blaser MF.
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