



12 **ABSTRACT**

13 Quantifying relationships between species richness and single environmental factors is challenging  
14 as species richness typically depends on multiple environmental factors. Recently, various methods  
15 have been proposed to tackle this challenge. Using a dataset comprising field observations of  
16 grassland vegetation and measured pH values, we compared three methods for deriving species  
17 richness response curves. One of the methods estimates species richness close to the maximum  
18 species richness observed at the sites, whereas the other two provide estimates of the potential  
19 species richness along the environmental gradient. Our response curves suggest that potential  
20 species richness of grasslands is slightly more sensitive to acidification than realized plant species  
21 richness. However, differences in corresponding environmental quality standards (EQS) for  
22 acidification were small compared to intrinsic spatial differences in natural soil pH, indicating that  
23 natural background values are more important to consider in the derivation of EQS for pH than  
24 methodological differences between the three approaches.

25

26 **Capsule:** We compared three methods to derive field-based species sensitivity distributions (f-SSDs)  
27 from presence-absence data.

28

29 **Key words:** Survey data; pH; Stressor-Response Relationships; Species Sensitivity Distributions  
30 (SSDs); Environmental Quality Standards (EQS)

31

## 32 INTRODUCTION

33 Environmental factors that determine species distribution patterns and species richness are of  
34 primary interest to nature conservation (Pausas & Austin, 2001). Quantifying the influence of  
35 individual factors on species communities in a systematic way can help to improve our  
36 understanding and predictive ability of biodiversity patterns, derive environmental quality  
37 standards, and underpin abatement priorities (Latour and Reiling, 1993; Latour et al., 1994; Van  
38 Goethem et al., 2013; Wamelink et al., 2013). However, species distributions are typically dependent  
39 on multiple environmental factors, including both abiotic and biotic drivers (Pulliam, 2000; Schipper  
40 et al., 2014; Soberón, 2007). As confounding environmental factors generally result in considerable  
41 scatter among species richness observations, it is not straightforward to extract relationships  
42 between species richness and single factors from field data (Cade and Noon, 2003; Van den Brink et  
43 al., 2002).

44 Recently, various methods have been proposed to tackle this challenge (Leung et al., 2005; Struijs et  
45 al., 2011; Kefford et al., 2011; Iwasaki & Ormerod, 2012; Azevedo et al., 2013; Cormier & Suter II,  
46 2013). Most of these methods are based on occurrence data (e.g. presence-absence data), which are  
47 generally more readily available than abundance data (Pearce & Boyce, 2006; Potts & Elith, 2006).  
48 One method is to relate site-specific observations of the number of species present to a particular  
49 environmental variable with quantile regression (Iwasaki & Ormerod, 2012). Most regression  
50 techniques relate changes in the mean of a response variable to one or more explanatory variables.  
51 With quantile regression, any part of the distribution of a variable can be used as response (Cade  
52 and Noon 2003). Quantile regression based on one of the upper boundaries of the response variable  
53 distribution (e.g. the 0.95 or 0.99 quantile) is expected to show the constraints imposed by the  
54 explanatory environmental variable of concern (Iwasaki & Ormerod, 2012; Lancaster and Belyea,  
55 2006). A second method is to assess the number of species present within regular intervals along a  
56 particular environmental gradient by pooling multiple samples per interval ('pooled samples

57 method'). The number of species per interval is then assessed either by simply counting the number  
58 of unique species across all samples within the interval (Struijs et al., 2011) or by establishing a  
59 species accumulation curve (SAC) per interval, thus correcting for potential differences in the  
60 number of samples between the intervals (Kefford et al., 2011). With a third method, observations  
61 of multiple species across multiple samples are used to first establish species-specific occurrence  
62 ranges, represented by the minimum and maximum values of the environmental variable of concern  
63 where the species has been observed. These occurrence ranges are then stacked across the species  
64 to arrive at an estimate of species richness ('occurrence range method'; Verbrugge et al., 2012,  
65 Azevedo et al., 2013, Cormier et al., 2013b).

66 Given the differences in approach, these three methods are expected to yield different species  
67 richness estimates, reflecting differences in potential and realized species richness. Potential species  
68 richness refers to the species that could occur at a specific site, while realized species richness refers  
69 to the species that actually occur there (Jiménez-Valverde et al., 2008). By modelling an upper  
70 quantile of the distribution of species richness actually observed at the sampling sites, the quantile  
71 regression method yields an estimate of the maximum species richness that may be realized at a  
72 particular location with a given pH. In contrast, the other two methods yield species richness  
73 estimates representing the pool of plant species corresponding with a given pH, i.e., the potential  
74 species richness. Species richness typically increases with an increasing number of samples (Kefford  
75 et al., 2011). Hence, aggregating observations from multiple sampling sites at each given interval  
76 along a particular environmental gradient, as is done in the pooled samples method, is expected to  
77 yield considerably higher values of species richness than can be observed at specific sampling sites  
78 (Kefford et al., 2011). The occurrence range method, finally, is expected to yield the highest  
79 estimates of species richness, by aggregating the species occurrences over the full environmental  
80 gradient rather than for each given interval separately.

81 The goal of this paper was to compare the three methods by applying them to the same species-  
82 environment dataset and quantifying the differences in the resulting species richness response  
83 curves. The dataset comprises presence-absence observations of terrestrial plant species along a  
84 gradient of soil pH measurements (pH 3-10) collected from 4412 sampling sites of grassland  
85 vegetation across the Netherlands (Wamelink et al., 2012). The methods were compared by  
86 quantifying the shapes of the response curves (magnitude, width) along the pH gradient.  
87 Furthermore, we compared the methods in terms of environmental quality standards, i.e. the pH  
88 levels corresponding with a predefined relative reduction in species richness (Van Straalen &  
89 Denneman, 1989; Posthuma et al., 2002). To achieve this we converted the species richness  
90 estimates to relative values with a maximum of 100%, thus obtaining field-based species sensitivity  
91 distributions (f-SSDs), i.e., empirical distributions describing interspecies variation in sensitivity to a  
92 particular environmental variable.

## 93 **METHODS**

### 94 **Species richness response curves**

#### 95 *Quantile regression*

96 The quantile regression method to estimate species richness along the pH gradient was based on  
97 Cade & Noon (2003). In our study, three models were constructed at the 95% quantile (Visser &  
98 Sasser, 2009): a linear model ( $y = \theta_0 + \theta_1 \cdot x$ ), a Gaussian model ( $y = \theta_0 + \theta_1 \cdot x + \theta_2 \cdot x^2$ ) and a baseline  
99 model where species richness is estimated by a constant (i.e., an intercept-only model). The most  
100 parsimonious model was selected based on the Bayesian Information Criterion (Lee et al., 2013). The  
101 different models were also constructed for the 97.5% and 99% quantiles to assess the influence of  
102 the quantile selection on the species richness estimates. The quantile regression was performed  
103 with the quantreg package in R (Koenkers et al., 2013).

#### 104 *Pooled samples method*

105 With the pooled samples method (Kefford et al., 2011), we derived species accumulation curves  
106 (SACs) for each interval  $i$  along the pH gradient. The SACs were derived using a resampling  
107 rarefaction method (100 times) that calculates the mean number of species observed ( $SR_{est}$ ) in 1 to  $n$   
108 samples, where  $n$  is the total number of samples pooled. The  $SR_{est}$  in  $k$  samples,  $SR_{est}(k)$ , is the mean  
109 number of species estimated in  $k$  samples. The  $SR_{est}(inf)$  is the mean number of species where one  
110 added sample leads to a maximum increase of less than one species (Verberk et al., 2006). For each  
111 interval  $i$  we considered the  $SR_{est}(inf)$  as an estimate for  $SR_{i,j}$  (Kefford et al., 2011). The intervals  $i$   
112 were set at 0.1 pH unit, so that there were enough observations in each interval to derive  $SR_{est}(inf)$   
113 (Table S2). The SACs were extrapolated up to a maximum of 5 times to ensure that  $SR_{est}(inf)$  could  
114 be estimated for all intervals (Colwell, 2012). As a sensitivity check the response curves were also  
115 derived based on 50, 20 and 1 samples. The SACs were determined using the computer software  
116 EstimateS 7.5.1 (Colwell, 2004).

#### 117 *Occurrence range method*

118 Following (Azevedo et al. 2013a), we defined the occurrence range for each species as the range  
119 between minimum and maximum pH values corresponding to the occurrence of that species as  
120 observed in the field. A species was considered to be absent at pH values outside this range, and  
121 potentially present at values inside its occurrence range. Species richness ( $SR_i$ ) was computed as the  
122 number of species potentially present at each pH interval  $i$  as

$$123 \quad SR_i = \sum_s O_{s,i} \quad (Eq. 1)$$

124 where  $O_{s,i}$  is the occurrence of each species  $s$  at pH interval  $i$ , with  $O = 0$  when the pH value is outside  
125 a species' occurrence range and  $O = 1$  if the pH value is within its occurrence range. The intervals  $i$   
126 for pH were set at 0.1. To assess the sensitivity of  $SR$  to changes in occurrence ranges, the species

127 occurrences were also derived based on the 5<sup>th</sup> and 95<sup>th</sup> and 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles of the pH  
128 values corresponding to the field occurrence of that species.

## 129 **Dataset**

130 The ecological conditions (EC) database compiled by Wamelink et al. (2012) was used in this study.  
131 This database comprised vegetation relevés from the Netherlands, each accompanied by a  
132 measured value of at least one abiotic soil parameter. The database contained 5243 grassland  
133 relevés with a measured pH value, covering the period from 1936 to 2011 (Table 1). pH values were  
134 measured in H<sub>2</sub>O extract and ranged from 3.0 to 10.1. Several relevés were part of a time series: the  
135 dataset included 141 sites where a relevé was made at least twice. To remove potential confounding  
136 influences of temporal autocorrelation, we included only the most recently recorded relevés from  
137 each time series in the dataset. This led to a decrease in the number of relevés of 5243 to 4412. The  
138 vegetation relevés were made according to the Braun-Blanquet method and followed the  
139 syntaxonomical classification of Schaminée et al. (1995)(Braun-Blanquet, 1921). In total 1321 species  
140 were recorded in the relevés. More details regarding the EC database can be found in Wamelink et  
141 al. (2012).

## 142 **Estimated vs. observed species richness**

143 We compared the estimated species richness ( $SR_{est}$ ) with the observed species richness ( $SR_{obs}$ ) over  
144 the relevés by deriving the average relative difference over the pH gradient as

$$145 \quad RD_{est-obs} = \frac{1}{N_i} \sum_i \frac{SR_{est,i} - \overline{SR_{obs,i}}}{\overline{SR_{obs,i}}} \quad (\text{Eq. 2})$$

146 where  $SR_{est,j}$  represents the species richness estimated for interval  $i$  and  $N_j$  is the total number of  
147 intervals.

148 **Field-based species sensitivity distributions (f-SSDs) and environmental quality standards (EQS)**

149 We derived a field-based species sensitivity distribution (f-SSD), an approach developed in the field  
150 of ecotoxicology, from each of the three species response curves. To that end, the estimated species  
151 richness was transformed into a zero-to-one measure, the relative species richness (r-SR), as

$$152 \quad rSR_i = \frac{SR_i}{SR_{\max}} \quad (\text{Eq. 3})$$

153 where  $SR_{\max}$  for a given method represents the highest species richness estimated in any interval  $i$   
154 along the pH gradient. The maximum r-SR (i.e.,  $r-SR = 1$ ) is obtained if the species richness in a  
155 particular interval  $i$  equals  $SR_{\max}$ , while  $r-SR = 0$  represents the complete absence of species. The  
156 resulting f-SSDs thus represent changes in species richness in relation to the pH gradient, relative to  
157 the highest species richness estimated for a particular pH level in the study area. Subsequently, for  
158 the pooled samples and occurrence range methods, we applied least squares regression to the r-SR  
159 estimates to obtain an explicit function of r-SR in relation to pH. Next, we used the resulting  
160 functions to derive environmental quality standards (EQS). We defined the EQS as the pH value  
161 corresponding with a 5% reduction of the species richness due to acidification (Posthuma et al.,  
162 2002).

163 **RESULTS**

164 **Species richness response curves**

165 The response curves based on the quantile regression, pooled samples and occurrence range  
166 methods all showed a unimodal response along the pH gradient (pH 3-10) (Figure 1). Optimum pH  
167 values were found in the range of 6.1-6.5 (Table 2). The response curves differed in their width and  
168 relative amplitude, where width is defined as the pH range at half  $SR_{\max}$  and relative amplitude as  
169 the relative difference between maximum and minimum species richness estimated along the pH



170 gradient (Table 2). The widths ranged from 4.7 units for the occurrence range method to 6.9 units  
171 for the quantile regression method. The relative amplitude ranged from 0.56 for the quantile  
172 regression method to 1.0 for the occurrence range method.

### 173 **Comparison with observed species richness**

174 The maximum estimated species richness was 50 for the quantile regression method, 590 for the  
175 pooled sample method and 702 for the occurrence range method (Table 2). The response curve  
176 based on the quantile regression method followed the highest observed species richness in the field,  
177 whereas the pooled samples and occurrence range methods estimated much higher SR (Figure 1).  
178 The average relative difference between  $SR_{est}$  and  $SR_{obs}$  ranged from 1.1 for the quantile regression  
179 method to 17.6 for the occurrence range method (Table 2). Per method, the average observed SR  
180 and estimated SR per interval are given in Table S2. Based on the average relative difference  
181 between the estimated and observed SR, the quantile regression method is placed on the left of the  
182 gradient from realized to potential species richness, whereas the occurrence range and pooled  
183 samples methods are placed towards the right (Figure 2).

### 184 **Field-based species sensitivity distributions and environmental quality standards**

185 Field-based species sensitivity distribution (f-SSDs) for the quantile regression, pooled sample and  
186 occurrence range methods are given in Figure 3. The environmental quality standards (EQS), i.e., the  
187 pH levels corresponding with a 5% reduction in species richness in case of acidification, were most  
188 stringent for the occurrence range method, followed by the pooled samples and quantile regression  
189 methods (Figure 4). However, the difference in EQS between the three methods was 0.3 pH unit at  
190 maximum.

### 191 **Sensitivity analysis**

192 Sensitivity of the quantile regression method to changes of the chosen quantile was tested by  
193 comparing response curves based on the 95<sup>th</sup>, 97.5<sup>th</sup> and 99<sup>th</sup> quantiles (Figure S1; Table S1). The  
194 response curves gave similar results, with a maximum pH of 6.5 irrespective of the quantile. The  
195 estimated SR in the pooled sample method depends on the number of samples used to derive the  
196 SACs and ranged from a maximum SR of 32 for one sample to a maximum SR of 601 for an infinite  
197 number of samples (Figure S2). At the extremes of the pH range, however, the differences in  
198 estimated SR are smaller, ranging from 13 to 122 at pH 3.5 and from 12 to 123 at pH 9. In the  
199 occurrence range method the width and maximum estimated SR is determined by the percentile  
200 that is used to derive the species occurrence ranges (Figure S3). The width ranged from 4.6 for the  
201 100<sup>th</sup> to 3.6 for the 90<sup>th</sup> percentile. The maximum estimated SR ranged from 633 for the 90<sup>th</sup> to 721  
202 for the 100<sup>th</sup> percentile.

## 203 **DISCUSSION**

### 204 **Interpretation**

205 Each of our response curves suggests a unimodal relationship between the species richness of  
206 grassland vegetation and pH (Figure 1). The unimodal response is in line with the results of other  
207 studies, with comparable optimum pH and shape of the curve (Azevedo et al., 2013; Chytrý et al.,  
208 2010; Olsson et al., 2009; Wamelink et al., 2005). This suggests that the relationship between species  
209 richness and pH was successfully extracted from the field data. We derived the response curves  
210 specifically for grassland vegetation, as different vegetation types respond differently to changes in  
211 pH (Wamelink et al., 2005). Response curves with multiple optima may result from a dataset  
212 including multiple vegetation types (Figure S4), suggesting that response curves are preferably  
213 derived per vegetation type. However, response curves based on species richness do not account for  
214 changes in species composition that may occur within a vegetation type, because the number of  
215 species may remain the same along a particular environmental gradient, whereas species

216 composition may change due to species replacements. Such species replacements may explain why  
217 the pooled samples method does not reveal major changes in species richness at intermediate pH  
218 levels (Figure 1). Species replacements along the pH gradient may also explain why the maximum  
219 number of species as obtained with the occurrence range method (702) is smaller than the total  
220 number of species in the dataset (1321). Apparently, there are no pH values that are within the  
221 occurrence range of all the species, and the gradient in pH values is large enough to encompass  
222 multiple non-overlapping tolerance ranges of individual species.

223 As expected, the quantile regression method estimated species richness close to the maximum  
224 species richness observed at the sampling sites, i.e. the maximum realized species richness (Figure 1;  
225 Table 2). The maximum realized species richness was around 50 at intermediate pH values. Because  
226 the quantile regression curve is directly derived from the species richness observed in the field sites,  
227 this method in particular may be sensitive to the size of the relevés, as bigger plot sizes may lead to  
228 higher species richness. Furthermore, the quantile regression method may be particularly sensitive  
229 to underestimated species richness due to false absence records, for example because some plant  
230 species may not have germinated yet at the moment the relevé was recorded. However, the surface  
231 area of the relevés is chosen to obtain a representative picture of the species composition and  
232 richness of the respective vegetation type, and sites are generally visited during the growing season,  
233 when most species are present and visible (Schaminee et al., 1995).

234 At intermediate pH values, the potential species richness as derived with the pooled samples and  
235 occurrence range methods was about 10 to 14 times larger than the maximum realized species  
236 richness (Figure 1). Potential species richness estimates were higher for the occurrence range  
237 method than for the pooled samples method. This difference is found because in the occurrence  
238 range method a species is assumed to occur anywhere between its minimum and maximum pH  
239 value, irrespective whether it was actually observed at the pH values in between, whereas in the

240 pooled samples method a species needs to be actually observed in a particular pH interval in order  
241 to contribute to the potential species richness.

242 The shapes of our response curves suggest that acidification would result in greater reductions in  
243 potential than in maximum realized plant species richness (Figure 1). This result is in line with two  
244 recent studies that concluded that large-scale declines of species richness are not necessarily  
245 accompanied by biodiversity loss at local scales (Dornelas et al., 2014; Vellend et al. 2013).

### 246 **Management implications**

247 For each response curve method we derived a field-based Species Sensitivity Distribution (f-SSD)  
248 (Figure 3). SSDs are typically derived for toxicants, generally based on a limited number of species  
249 tested in laboratory exposure experiments (Van Straalen & Denneman, 1989). To conduct laboratory  
250 experiment for all possible combinations of stressors and species, however, is almost impossible  
251 simply because there are so many (Azevedo et al., 2013; Cormier & Suter II, 2013a; Kefford et al.,  
252 2012). SSDs based on field observation have therefore been proposed instead, as these include the  
253 actual species pool and relevant environmental stressors of a particular area (Leung et al., 2005).  
254 The resulting f-SSDs can be used (1) to derive environmental quality standards for a particular  
255 environmental factor, and (2) to estimate relative changes in species richness along a specific  
256 environmental gradient (Posthuma et al., 2002). However, in contrast to anthropogenic toxicants,  
257 pH is a natural environmental factor, with varying natural background levels and ecological  
258 communities adapted to these levels (Wamelink et al., 2005). In order to account for this natural  
259 variation, we derived EQS based on a 5% reduction of the species richness corresponding with a  
260 given natural background pH (Figure 4), rather than a 5% reduction of the overall maximum species  
261 richness, as is common practice in EQS setting. EQS were slightly more stringent for potential than  
262 for maximum realized species richness, thus reflecting that acidification would result in larger  
263 declines of the former. However, differences in EQS between the three methods were only small and

264 EQS varied mainly in relation to the natural background pH. Hence, in the derivation of EQS for pH it  
265 is much more important to consider intrinsic spatial differences in soil pH than methodological  
266 differences between f-SSD approaches.

## 267 **ASSOCIATED CONTENT**

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

269 Figure S1: Field-based stressor response curves based on the quantile regression method derived the  
270 95<sup>th</sup>, 97.5<sup>th</sup> and 99<sup>th</sup> quantiles.

271 Figure S2: Field-based stressor response curves based on the pooled sample method derived with  
272 infinite, 50, 20 and 1 samples.

273 Figure S3: Field-based stressor response curves based on the occurrence range method derived with  
274 different percentiles.

275 Figure S4: Field-based response curves for pH based on the pooled sample method derived for  
276 grasslands, forests and heathland.

277 Figure S5: Field-based stressor response curves based on the pooled sample method with the 5<sup>th</sup> and  
278 95<sup>th</sup> confidence intervals.

279 Table S1: The regression coefficients for the regression models based on the 95<sup>th</sup>, 97.5<sup>th</sup> and 99<sup>th</sup>  
280 quantile.

281 Table S2: The number of relevés, average observed SR and estimated SR per interval for each  
282 method.

## 283 **ACKNOWLEDGEMENTS**

284 We thank Zoran Steinmann (Radboud University) for helping out with the R software, Stephan  
285 Hennekens (Wageningen University) for helping with the vegetation relevés, and all people  
286 contributing, collecting samples and providing data to the Ecological Conditions database (EC).

287

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402

403 **Table 1. Characteristics (Mean, SD, Median, Min, Max and various percentiles) of the measured pH values**  
 404 **and species richness for 4412 relevés.**

	<b>Mean</b>	<b>SD</b>	<b>Median</b>	<b>Min</b>	<b>0.025</b>	<b>0.25</b>	<b>0.75</b>	<b>0.975</b>	<b>Max</b>
<b>pH values</b>	5.7	1.3	5.6	3.0	3.8	4.7	6.4	7.9	10.1
<b>Species richness</b>	25	12	24	1	5	14	31	48	73

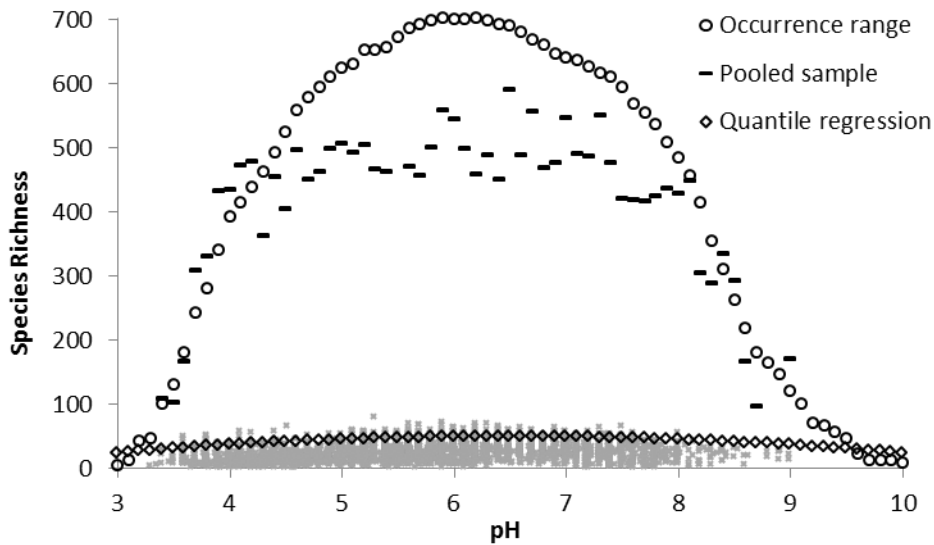
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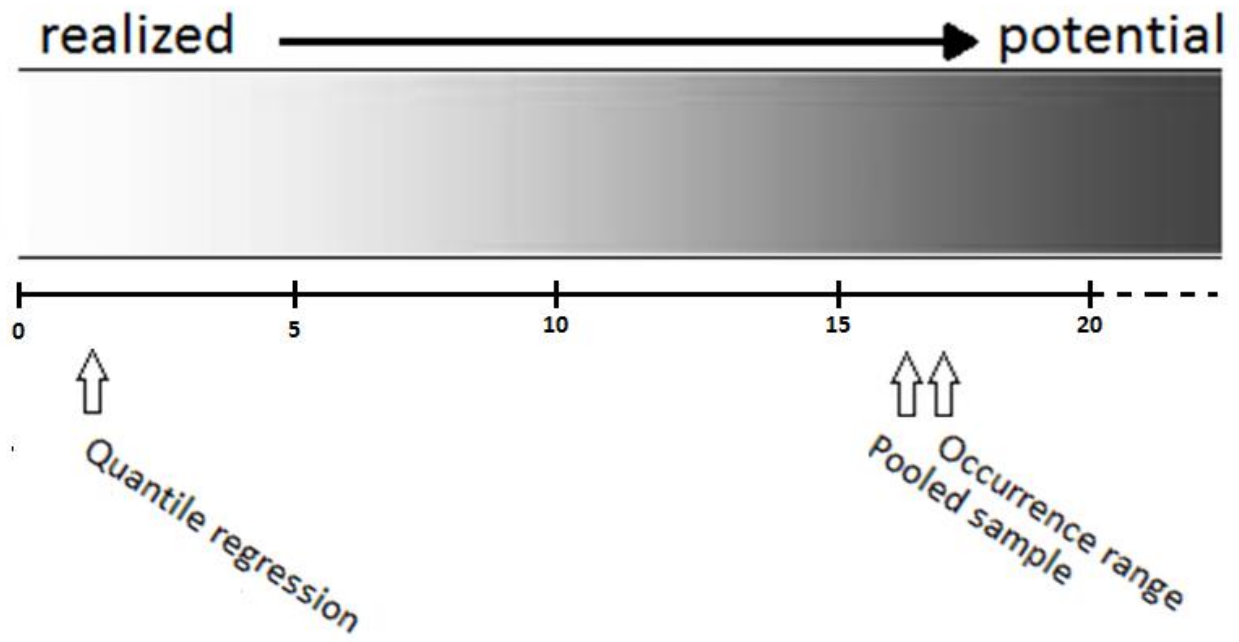
407 Table 2. Optimum pH ( $pH_{max}$ ), the width at 0.5 SR (width  $SR_{0.5}$ ) and relative amplitude of the species richness  
 408 response curves, maximum SR ( $SR_{max}$ ), average relative difference between the estimated SR and the  
 409 observed SR ( $RD_{est-obs}$ ) for each of the response curve methods.

	Quantile Regression	Pooled Samples	Occurrence Range
$pH_{max}^*$	6.5	6.1	6.3
Width $SR_{0.5}^*$	6.9	5.1	4.7
Relative amplitude <sup>*</sup>	0.56	0.76	1.00
$SR_{max}$	50	590	702
$RD_{est-obs}$	1.1	16.0	17.6

410 <sup>\*</sup> $pH_{max}$ , width  $SR_{0.5}$  and relative amplitude based on the f-SSDs



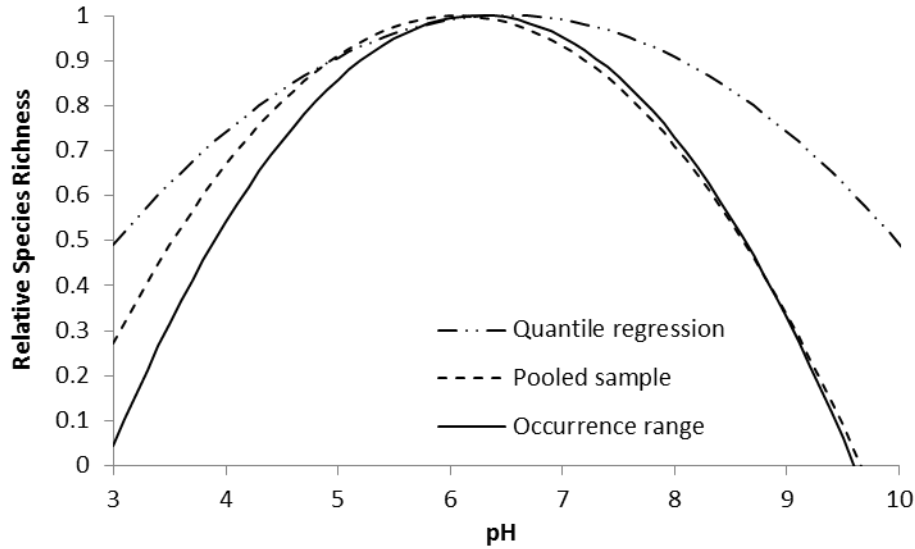
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 412 Figure 1. Field-based species richness response curves for pH derived with the quantile regression method,  
 413 pooled samples method and the occurrence range method. Observed SR is plotted in gray. In the quantile  
 414 regression method the Gaussian model was selected as the most parsimonious model based on the 0.95  
 415 quantile (Table S1; Figure S1). Confidence intervals for the SR estimates derived with the pooled sample  
 416 method can be found in Figure S2.



417

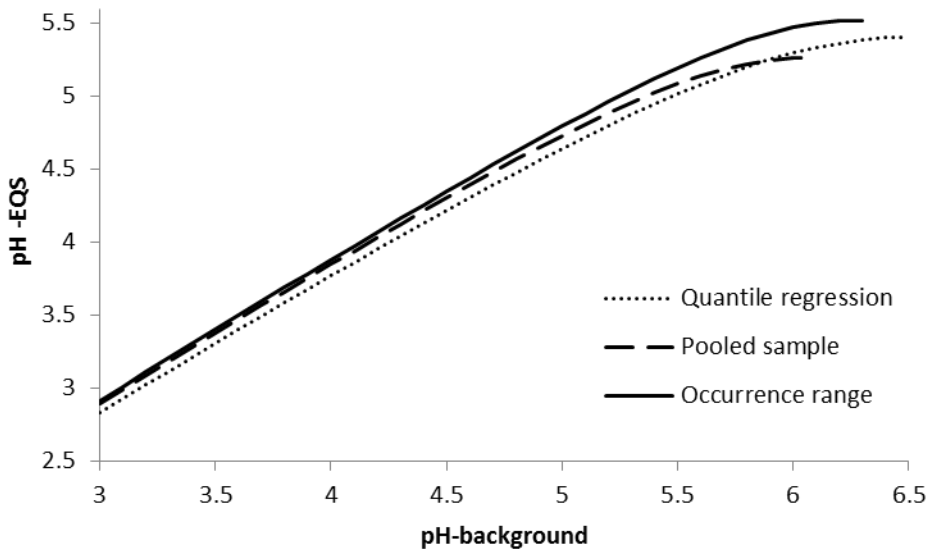
418 Figure 2. Representation of the three response curve methods on a gradient from realized to potential  
 419 species richness (adapted from Jiménez-Valverde et al., 2008). Numbers on the axis indicate the average  
 420 relative difference between the estimated and average observed SR for each of the response curve  
 421 methods.

422



423

424 **Figure 3.** f-SSDs for the relative species richness (r-SR) along the pH gradient for the quantile regression ( $y = -$   
 425  $0.75(-1.13- -0.31)+0.54(0.32-0.74)x-0.04(-0.06- -0.03)x^2$ ), pooled sample ( $y = -1.94(-2.32- -1.22)+0.94(0.65-$   
 426  $1.12)x-0.08(-0.10- -0.05)x^2$ ), and occurrence range methods ( $y = -2.55(-3.16- -1.89)+1.12(0.94-1.32)x-0.03(-$   
 427  $0.10- -0.01)x^2$ ). The 95<sup>th</sup> percentile confidence intervals of the regression coefficients are given between  
 428 brackets.



429

430 **Figure 4.** Environmental quality standards for pH (pH-EQS) corresponding to the respective background  
 431 levels (pH-natural background) for each method.