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**Response to Čapek and Šantrůčková’s comment to
“Liming effects on microbial carbon use efficiency and
its potential consequences for soil organic carbon stocks”
[Soil Biology and Biochemistry 194: 109437]**

Julia Schroeder, Claudia Dămătîrcă, Tobias Bölscher, Claire Chenu, Lars
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2 Response to Čapek and Šantrůčková's comment to "Liming effects on
3 microbial carbon use efficiency and its potential consequences for
4 soil organic carbon stocks" [Soil Biology and Biochemistry 194:
5 109437]

6 Julia Schroeder¹, Claudia Dămătîrcă^{2,6}, Tobias Bölscher³, Claire Chenu³, Lars Elsgaard⁴, Christoph C.
7 Tebbe⁵, Laura Skadell¹, Christopher Poeplau¹

8 ¹ Thünen Institute of Climate-Smart Agriculture, Bundesallee 68, 38116 Braunschweig, Germany

9 ² University of Turin, Department of Agricultural, Forest and Food Sciences, Largo Paolo Braccini 2, 10095 Grugliasco TO, Italy

10 ³ Université Paris-Saclay, INRAE, AgroParisTech, UMR EcoSys, 22 place de l'Agronomie, 91120 Palaiseau, France

11 ⁴ Aarhus University, Department of Agroecology, Blichers Allé 20, 8830 Tjele, Denmark

12 ⁵ Thünen Institute of Biodiversity, Bundesallee 65, 38116 Braunschweig, Germany

13 ⁶ current address: Euro-Mediterranean Center on Climate Change (CMCC) Foundation, Division on Climate Change Impacts on Agriculture,
14 Forests and Ecosystem Services (IAFES), Via Igino Garbini 51, 01100 Viterbo, Italy

15

16 Julia Schroeder	julia.schroeder@thuenen.de	ORCID: 0000-0003-3625-104X
17 Claudia Dămătîrcă	claudia.damatirca@cmcc.it	ORCID: 0000-0003-1778-668X
18 Tobias Bölscher	tobias.bolscher@inrae.fr	ORCID: 0000-0001-5305-0616
19 Claire Chenu	claire.chenu@inrae.fr	ORCID: 0000-0001-9054-0489
20 Lars Elsgaard	lars.elsgaard@agro.au.dk	ORCID: 0000-0003-0058-7609
21 Christoph C. Tebbe	christoph.tebbe@thuenen.de	ORCID: 0000-0003-4861-0214
22 Laura Skadell	laura.skadell@thuenen.de	ORCID: 0000-0002-4789-8474
23 Christopher Poeplau	christopher.poeplau@thuenen.de	ORCID: 0000-0003-3108-8810

24

25 Corresponding author: Julia Schroeder
26 julia.schroeder@thuenen.de
27 +49 531 596-2723
28

29 We highly appreciate the effort of Čapek and Šantrůčková (2024), who in detail recalculated our
30 assessment of the possible underestimation of microbial respiration rates measured in alkaline soils
31 for analysing carbon use efficiency (CUE). Thanks to their comment, we were not only pointed to two
32 typographical mistakes in Eq. 1 provided in our original manuscript (Schroeder et al., 2024), but also
33 to an unfortunate error in unit conversion in our assessment provided in the supplemental information
34 along with the original manuscript. We corrected these errors in an erratum. Čapek and Šantrůčková
35 (2024) suggest that the potential underestimation of microbial respiration could affect our main
36 conclusion on the relationship between soil pH and microbial CUE. Here, we present a revised estimate
37 of the influence of the potential underestimation of microbial respiration on our results and argue that
38 our main conclusions still hold.

39 Background

40 In alkaline soils, microbial respiration rates can be underestimated if assessed by the increase in
41 headspace CO₂ over time and if the dissolution of CO₂ in soil water is not accounted for. This limitation
42 mainly affects soils at pH > 7, because the dissolution of CO₂ in water increases exponentially with

43 increasing soil pH (Table 1) due to shifts in the dissociation equilibria of inorganic C species. This effect
44 is unfortunately often ignored, but was addressed by us in the supplementary material.

45 In our study we investigated the effect of lime-induced soil pH increases at up to pH = 8.6 on microbial
46 CUE. This physiological parameter is calculated based on microbial respiration and growth (Eq. 1) and
47 defined as microbial biomass C produced (C_{Growth}) over the total uptake of C, approximated as the sum
48 of microbial biomass C produced and C respired ($C_{Respiration}$) (Manzoni et al., 2012).

$$49 \quad CUE = \frac{C_{Growth}}{C_{Growth} + C_{Respiration}}$$

50 Eq. 1

51 To account for the potential underestimation of microbial respiration we assessed how much
52 additional dissolved inorganic C (DIC) could be expected at a given pH, using a chemical equilibrium
53 software, i.e. Visual MINTEQ (see Supplementary data of Schroeder et al., 2024).

54

55 **Table 1** The distribution of CO₂ species between the gas phase and the soil solution (calculations based
56 on gas phase-pure water system) changes dramatically above a pH = 7 in the 0.1 mL water and 0.1 kPa
57 pCO₂ scenario. At high pH, a larger fraction of the microbially derived CO₂ would end up as DIC than as
58 CO₂ in the headspace. In comparison, the fraction of CO₂ ending in the gas phase at pH ≤ 7 would
59 account for over 98 % (Schroeder et al., 2024, Supplementary data).

pH	water scenario	pCO ₂ scenario	relative proportion C [%]	
			DIC	CO ₂ (gas)
5	0.1 mL	0.1 kPa	1	99
5,25	0.1 mL	0.1 kPa	1	99
5,5	0.1 mL	0.1 kPa	1	99
5,75	0.1 mL	0.1 kPa	1	99
6	0.1 mL	0.1 kPa	1	99
6,25	0.1 mL	0.1 kPa	1	99
6,5	0.1 mL	0.1 kPa	1	99
6,75	0.1 mL	0.1 kPa	2	98
7	0.1 mL	0.1 kPa	2	98
7,25	0.1 mL	0.1 kPa	4	96
7,5	0.1 mL	0.1 kPa	6	94
7,75	0.1 mL	0.1 kPa	10	90
8	0.1 mL	0.1 kPa	16	84
8,25	0.1 mL	0.1 kPa	26	74
8,5	0.1 mL	0.1 kPa	38	62
8,75	0.1 mL	0.1 kPa	52	48
9	0.1 mL	0.1 kPa	66	34
9,25	0.1 mL	0.1 kPa	78	22
9,5	0.1 mL	0.1 kPa	87	13

60

61 Correction of the potential underestimation of CO₂ production

62 In our original publication (Schroeder et al., 2024), an unfortunate erroneous conversion of units (i.e.
63 g to µg instead of ng) caused a miscalculation of three orders of magnitude as to the maximum amount
64 of CO₂ that could dissolve in the soil solution and form additional DIC. We therefore underestimated
65 the additional dissolved CO₂ at high pH (i.e., to be < 0.1 %) and incorrectly interpreted it as negligible.

66 Our here corrected calculations reveal a maximum relative underestimation of 108 % in the Versailles
67 *limed* treatment followed by an underestimation of 19 % in the Dürnast *High lime* treatment, while in
68 all other treatments the error was less than 7 % (Table 2). Our values are higher than those of Čapek
69 and Šantrůčková (2024), who calculated a maximum relative underestimation of 84 % for Versailles
70 *limed*. This is due to the different approaches used. Čapek and Šantrůčková (2024) calculated the
71 amount of DIC at a given pH and scenario based on the exact pH values according to their Eq. 6 and
72 Eq. 10. For simplicity, we used a set of scenarios using the software Visual MINTEQ, which uses the
73 standard database “thermo.vdb” for speciation calculations. The amount of DIC at different scenarios
74 was calculated for a range of pH values from 4.5 to 9.5 and we assigned the estimated maximum
75 relative underestimation of the pH closest to the mean soil pH to each treatment (Table 2). For
76 Versailles *limed* the mean treatment soil pH was 8.6, where the closest pH in Visual MINTEQ was 8.75.
77 Since the amount of DIC increases exponentially, the expected maximum relative error at pH 8.75 (i.e.,
78 108 %) is higher than that at pH 8.6 (i.e., 84 %). Another reason for the difference is that Čapek and
79 Šantrůčková (2024) for simplicity neglected the further dissociation of HCO₃⁻ to CO₃²⁻, while this step
80 was included in our Visual MINTEQ simulation.

81 **Table 2** Expected maximum relative underestimation of respiration rates for the different soils of the
82 study by Schroeder et al. (2024). Calculations using Visual MINTEQ are based on the estimation of
83 dissolved carbonate species in incubated soils (0.1 kPa partial pressure CO₂ represents an equivalent
84 of a respiration rate of 700 ng C g⁻¹ h⁻¹) as compared to the amount of CO₂ solved at an initial standard
85 gas CO₂ concentration of 350 ppm

Site	Treatment	Mean pH	Closest pH Visual MINTEQ	Expected maximum relative underestimation [%]	
				0.04 mL, 0.1 kPa	0.1 mL, 0.1 kPa
Jynde vad	control	4.5	5.0	0.21	0.51
	lime 4	5.7	5.75	0.24	0.60
	lime 8	6.9	7.0	0.95	2.38
	lime 12	7.3	7.5	2.58	6.46
Versailles	control	5.5	5.5	0.22	0.55
	limed	8.6	8.75	43.45	108.63
Dürnast	control	6.5	6.5	0.44	1.09
	Medium lime	7.4	7.5	2.58	6.46
	High lime	7.8	8.0	7.76	19.40

86

87 Consequences for the CUE and its soil pH relationship

88 Čapek and Šantrůčková (2024) indicate that proper correction of CO₂ estimates would likely change
89 the relationship between CUE and soil pH at high pH as shown in our Figure 3 of the original
90 manuscript. Assuming a linear increase in microbial growth along the pH gradient, and a constant
91 headspace partial pressure, they tentatively show in their Figure 2B that the corrected CUE may even

92 decline at high soil pH when accounting for the potential underestimation in microbial respiration
 93 (Čapek and Šantrůčková, 2024).

94 To assess the impact of a potential underestimation of microbial respiration on our main findings, we
 95 corrected $C_{\text{Respiration}}$ and derived CUE values corrected for the expected maximum relative
 96 underestimation according to Table 2 (see Supplementary data). An example of original and corrected
 97 values for the Versailles site, the site with the highest pH, is shown in Table 3.

98 **Table 3** Original and corrected results for Versailles control (*ctrl*) and *limed* samples. The corresponding
 99 expected maximum relative underestimation (EMRU) derived from the Visual MINTEQ simulation was
 100 assigned to each individual sample to correct microbial respiration and the resulting microbial carbon
 101 use efficiency (CUE)

ID	lime	pH	Closest pH Visual MINTEQ	EMRU [%]	$C_{\text{Respiration}}$ [ng g ⁻¹ soil h ⁻¹]	$C_{\text{Respiration}}$ corrected [ng g ⁻¹ soil h ⁻¹]	C_{Growth} [ng g ⁻¹ soil h ⁻¹]	CUE original	CUE corrected
VB 22	<i>ctrl</i>	5.44	5.5	0.55	226.87	228.13	1286.20	0.85	0.85
VB 30	<i>ctrl</i>	6.00	6.0	0.68	259.26	261.03	420.77	0.62	0.62
VB 32	<i>ctrl</i>	5.22	5.0	0.51	258.61	259.93	885.21	0.77	0.77
VB 34	<i>ctrl</i>	5.53	5.5	0.55	232.91	234.20	455.78	0.66	0.66
VB 26	<i>limed</i>	8.63	8.5	60.76	118.29	190.16	1236.71	0.91	0.87
VB 31	<i>limed</i>	8.57	8.5	60.76	183.72	295.35	652.67	0.78	0.69
VB 39	<i>limed</i>	8.55	8.5	60.76	170.35	273.85	1068.63	0.86	0.80
VB 40	<i>limed</i>	8.65	8.75	108.63	188.42	393.12	1189.23	0.86	0.75

102

103 The relative error in CUE is smaller because CUE is calculated from two C fluxes, i.e. $C_{\text{Respiration}}$ and C_{Growth}
 104 (Eq. 1). At our field sites, CUE was high so that $C_{\text{Respiration}}$ was a much smaller C flux than C used for
 105 microbial growth (i.e., C_{Growth}). Due to this fact, CUE estimates were substantially less influenced by
 106 expected maximum relative underestimation of respiration than the estimates of respiration
 107 themselves. In the case of Versailles, C_{Growth} is on average 3.5 times larger than $C_{\text{Respiration}}$, so that even
 108 larger relative changes in $C_{\text{Respiration}}$ do not strongly affect the CUE. It should however be pointed out
 109 that the relative underestimation of microbial respiration has considerably larger effects in soils with
 110 lower CUE, when the $C_{\text{Respiration}}$ flux dominates over C_{Growth} (Figure 1; Bölscher et al. 2024).

111 --- Approximate position Figure 1---

112 Figure 2A shows that, in alkaline soils, corrected CUE values are lower than depicted in the original
 113 data. CUE values were, in absolute values, 4 %, 7 % and 8 % reduced by the correction in the highest
 114 lime treatments at Jyndevad, Versailles and Dürnast, respectively. After the correction, the correlation
 115 between CUE and soil pH is no longer significant at Dürnast ($p = 0.132$) (Figure 2A). However, the
 116 overall relationship still follows a quadratic function ($p = 0.006$), explaining 25 % of variation in CUE as
 117 compared to 36 % for the original data (Figure 2B). From this relationship it can be concluded that at
 118 a given site, microbial CUE is likely to be reduced by a liming-induced pH increase at low pH, and
 119 increased at high pH, which was also the conclusion in the original contribution (Schroeder et al., 2024).
 120 For clarification, z-transformation excludes site-specific variation enabling to investigate the general
 121 relationship between CUE and soil pH. The extent of CUE changes along the pH gradient can thus vary
 122 in their intensity, depending on the site.

123 --- Approximate position Figure 2---

124 In our original manuscript, we presented that the microbial growth to soil pH relationship does follow
125 a quadratic function, while microbial respiration has a peak at near neutral soil pH following an
126 optimum curve. The relationship between CUE and soil pH is the outcome of the combined figures.
127 Correction of $C_{\text{Respiration}}$ for the expected maximum relative underestimation (0.1 mL, 0.1 kPa) results in
128 a linear increase in microbial respiration along the pH gradient ($p = 0.037$) (Figure 3). In their
129 simulation, Čapek and Šantrůčková (2024) assume that microbial growth increases linearly along the
130 pH gradient, resulting in a unimodal relationship with maximum CUE at pH 7.5. However, our Figures 2
131 and 3 suggest that this is not the case: 1) The quadratic relationship between CUE and soil pH is still
132 evident (Figure 2B), and 2) microbial growth does not linearly increase along the pH gradient.
133 Nevertheless, we agree that the potential underestimation of $C_{\text{Respiration}}$ induces some uncertainty in
134 the CUE and soil pH relationship at higher pH which cannot be neglected.

135 --- Approximate position Figure 3 ---

136 The presented correction can be considered conservative. To correct the $C_{\text{Respiration}}$ values, we used the
137 expected maximum relative underestimation derived from the 0.1 mL water and 0.1 kPa CO_2 partial
138 pressure scenario. A partial pressure of 0.1 kPa would be equivalent to a respiration rate of 700 ng C g^{-1}
139 soil h^{-1} . However, as can be seen in Table 3, even the corrected $C_{\text{Respiration}}$ rates did not exceed
140 $400 \text{ ng C g}^{-1} \text{ soil h}^{-1}$ at Versailles, which is at highest risk to be biased. Thus, the error estimation is
141 conservative in regards of the partial pressure assumption. The actual underestimation may be lower.
142 To be able to calculate the estimated maximum relative underestimation many assumptions had to be
143 made. For example, the system was simplified to consist only of pure water-gas phase. However, in
144 soil solution the solubility of CO_2 may be lower than in pure water, as indicated by lower Henry's law
145 constants with increasing salinity (Harned & Davis, 1943).

146 In Schroeder et al. (2024) we also investigated how laboratory CaCO_3 addition affected CUE. Since the
147 addition of CaCO_3 causes abiotic CO_2 emissions to the headspace, microbial- and lime-derived CO_2
148 sources need to be separated prior the calculation of $C_{\text{Respiration}}$. This was done using a two-pool mixing
149 model and $\delta^{13}\text{C}$ signatures of the lime and CO_2 in the headspace. We agree with Čapek and Šantrůčková
150 (2024) that assuming no isotopic fraction during CaCO_3 dissolution is a simplification. It however only
151 affected our lab liming measurements, whereas the field liming results are little affected by this
152 assumption, because CO_2 from CaCO_3 evolves in soils shortly after the application. At our field sites,
153 lime was last applied 1 (Versailles) to 8 years (Jyndevad) prior sampling. We assumed no further
154 contribution of lime to the CO_2 flux. In the field limed samples, we therefore ignore potential errors
155 due to CO_2 from lime. Our simplification is supported by results from the Versailles site. In Versailles,
156 two plots received CaCO_3 (plots 31 and 39) and two plots CaO (26 and 40). Only CaCO_3 leads to CO_2
157 formation from lime, and if liming would still add to the CO_2 flux to the headspace, it should only affect
158 plots 31 and 39. However, the CUE values were close to each other.

159 Conclusions

160 Interpretation of the CUE and soil pH relationship at high pH remains difficult, since the potential
161 underestimation of $C_{\text{Respiration}}$ adds to the already quite large uncertainty of the CUE values due to error
162 propagation along the assessed parameters (Pold et al., 2020; Bölscher et al., 2024). Particular caution
163 is required when examining and interpreting CUE and any other microbial parameter related to
164 respiration at high pH. Indeed, there is a need to raise - again (e.g. Martens, 1987; Sparling & West
165 1990) - the awareness of the limitations of current methods to quantify microbial respiration in alkaline
166 soils as the potential underestimation is rarely considered. As the current correspondence shows, it is

167 not trivial to correct for this type of underestimation. Considering the arguments by Čapek and
168 Šantrůčková and correcting our original data, we conclude that the main findings of this study hold.
169 The CUE and soil pH relationship likely follows a function between the two fitted curves, i.e. original
170 and corrected (Figure 2B).

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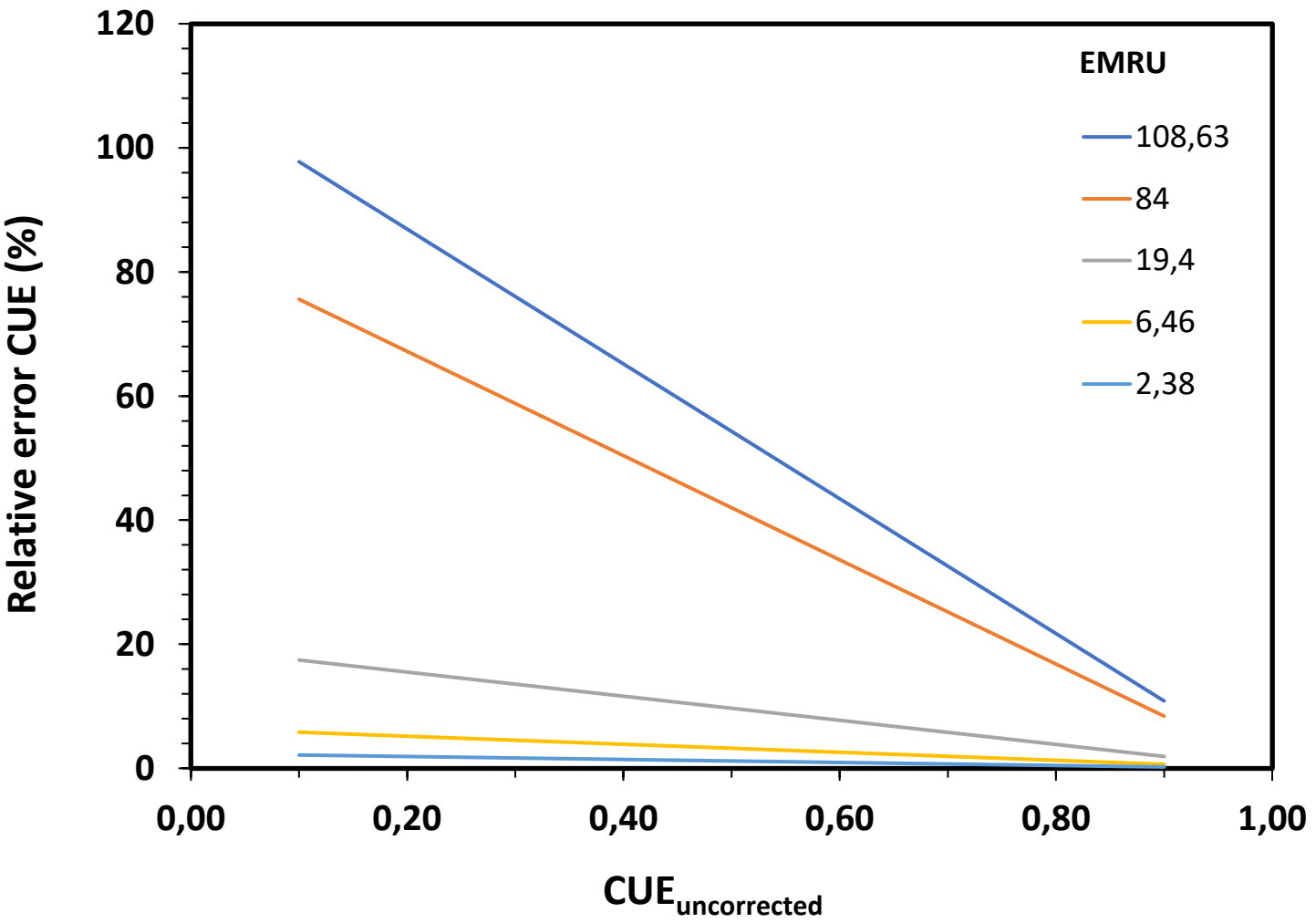
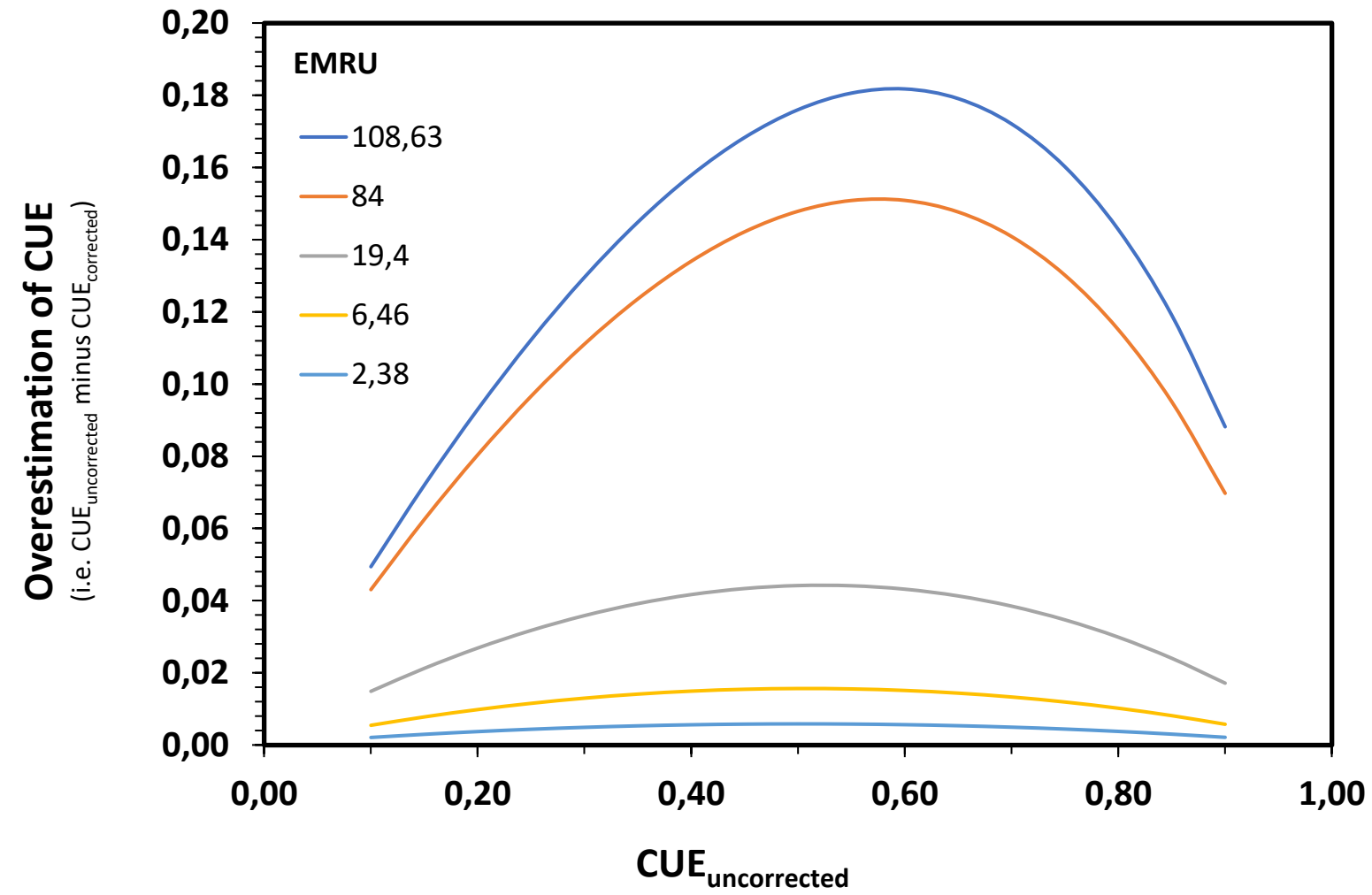
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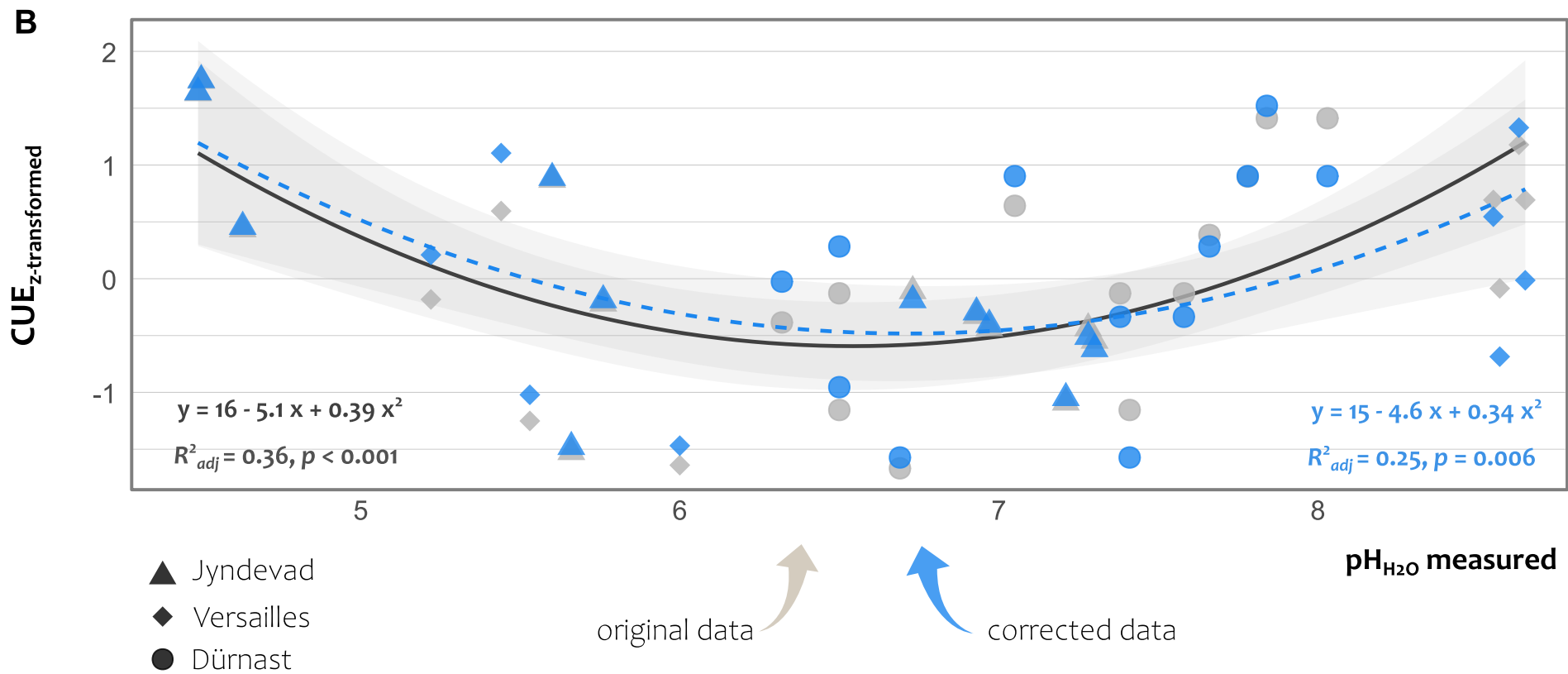
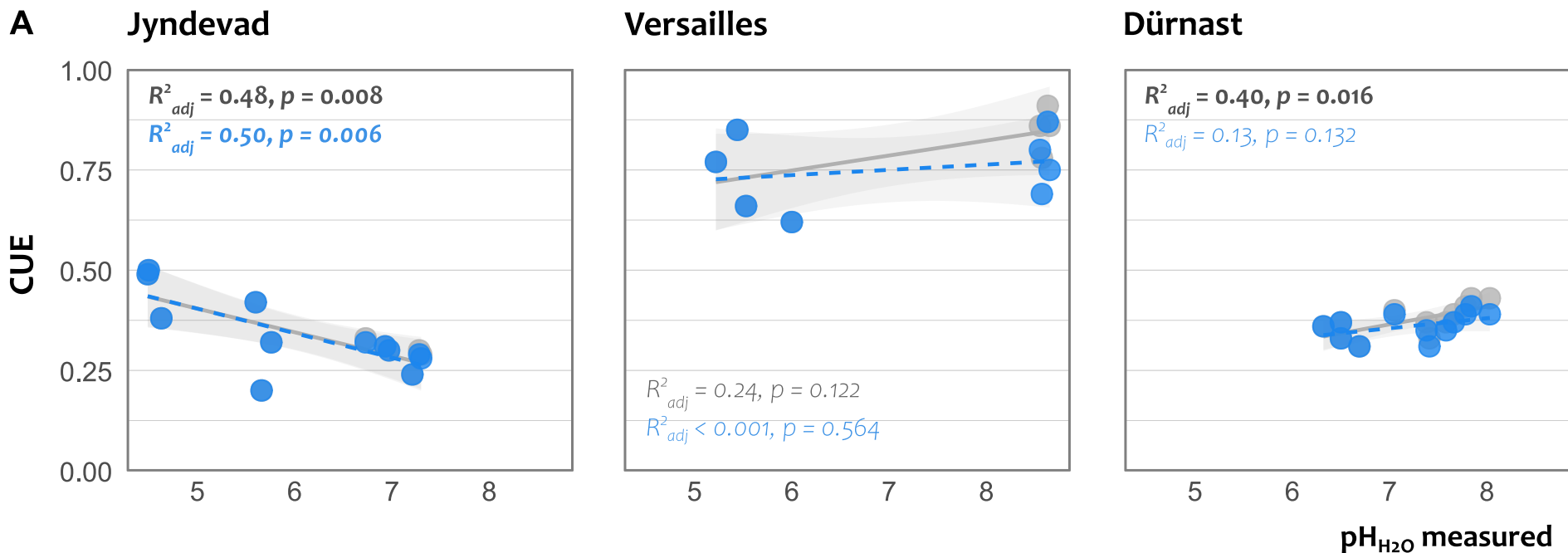
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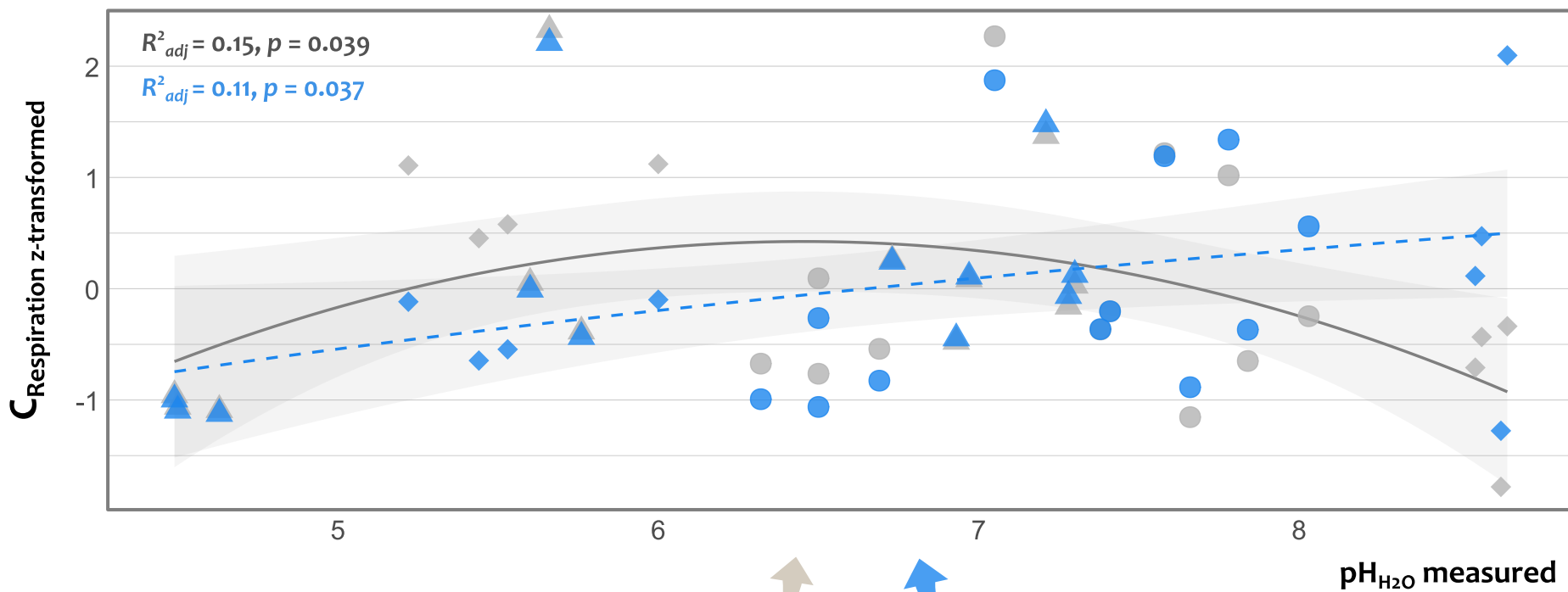
191 **Figure 1** The relative (A) and absolute (B) overestimation in CUE depends on the expected maximum
192 relative underestimation (EMRU in %) in microbial respiration and the CUE itself

193 **Figure 2** The impact of potential underestimation of microbial respiration in alkaline soils on microbial
194 carbon use efficiency (CUE) results: **A)** Absolute CUE values, and **B)** the general relationship between
195 CUE (z-transformed) and soil pH. Grey points depict the data for field liming as shown in Figure 3 of
196 the main text in Schroeder et al. (2024). Blue points present CUE values, which were corrected for the
197 estimated maximum relative underestimation of $C_{\text{Respiration}}$ (scenario 0.1 mL water, 0.1 kPa partial
198 pressure CO_2)

199 **Figure 3** The impact of potential underestimation of microbial respiration in alkaline soils on the overall
200 relationship between microbial respiration and soil pH (A). Grey points depict the data as shown in the
201 original manuscript. Blue points present respiration values which were corrected for the estimated
202 maximum relative underestimation in $C_{\text{Respiration}}$ (scenario 0.1 mL water, 0.1 kPa partial pressure CO_2).
203 B) shows the relationship between microbial growth and soil pH, which is not affected.

A**B**



A**B**