

Supplementary Information (SI) to

Title: Preferential substrate use decreases priming effects in contrasting treeline soils

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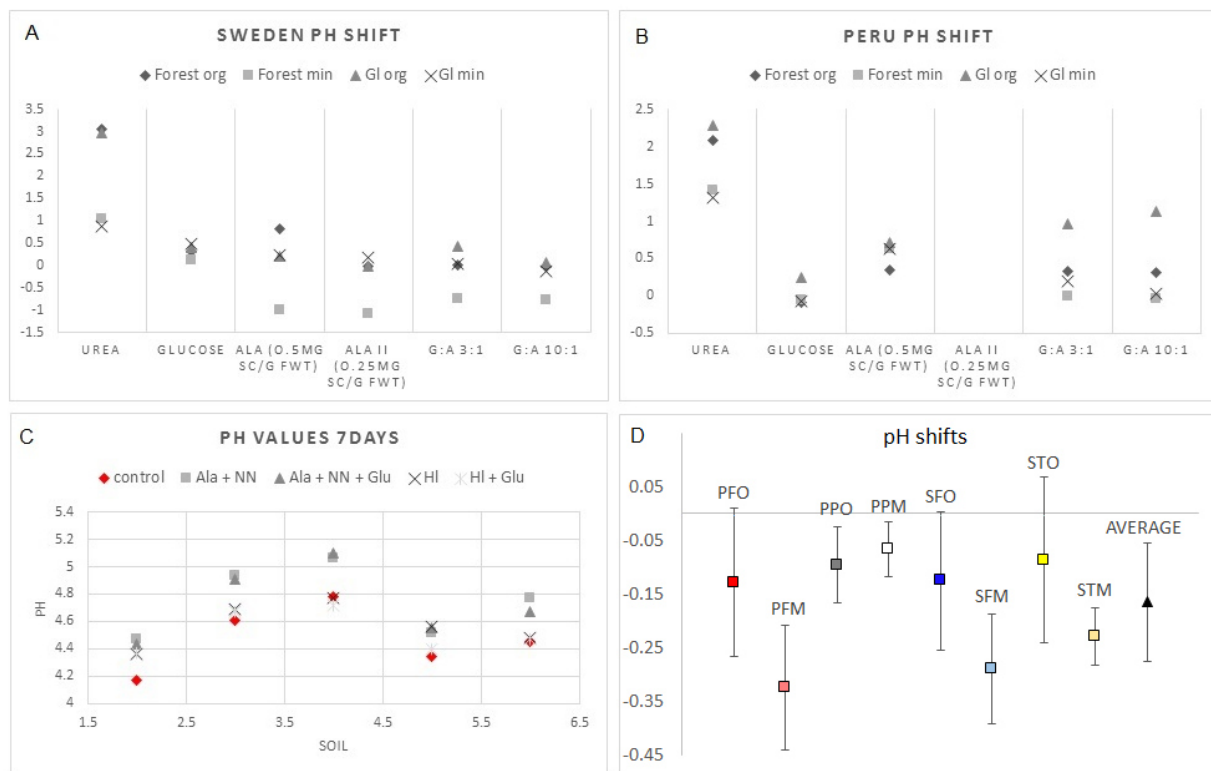
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Many laboratory soil incubations use substrates which significantly change the pH of the soil solution. Changing the pH of the soil solution directly effects the activity of soil microbes. Thus, conclusions drawn regarding the effect of the added substrate are impaired by these artificial changes of soil pH. We designed our experiment with the aim to minimize the bias of substrate-induced pH changes and therefore tested several of the commonly used substrates regarding their effect on soil pH before starting the experiment. In our test, we included the buffered Hoagland's No.2 nutrient solution (supplementary material 1)

Supplementary material 1: Composition of the nutrient solution used for the nitrogen treatments. The used Hoagland's Solution had a pH of 4.7 at room temperature.

Hoagland's No. 2 basal salt mixture (H2395 Sigma Aldrich)	Chemical formula	Component (mg litre⁻¹ stock solution)
Ammonium phosphate monobasic	(NH ₄) ₃ PO ₄	11.50
Boric acid	H ₃ BO ₃	0.29
Calcium nitrate	Ca(NO ₃) ₂	65.64
Cupric sulphate	CuSO ₄ • 5H ₂ O	0.01
Ferric tartrate	C ₁₂ H ₁₂ Fe ₂ O ₁₈ • 2H ₂ O	0.53
Magnesium sulphate	MgSO ₄ • H ₂ O	24.08
Manganese chloride	MnCl ₂ • 4H ₂ O	0.18
Molybdenum trioxide	MoO ₃	0.00
Potassium nitrate	KNO ₃	60.66
Zinc sulphate	ZnSO ₄ • 7H ₂ O	0.02
Total Nitrogen	N (various)	22.85 (0.022852 µg N µl ⁻¹)

We tested how the addition of urea, glucose, alanine at two concentrations, combinations of alanine and glucose, and Hoagland's No. 2 change soil pH (supplementary material 2). To test the substrate-induced shifts of soil pH for Hoagland's solution ("HI") and for Hoagland's with glucose ("HI+glu") we measured for the highest N addition (C:N = 7:1) on the replicates of each different soil type at the end of the incubation (Figure S2C). The used Hoagland's solution had a pH of 4.7 at room temperature. All pH was measured in water. In most cases substrate additions lowered soil pH, although occasional pH increases were observed. On average pH was reduced by 0.16 ± 0.11 , with the largest variance in organic soils and lowest absolute values in mineral forest soils from both countries (Figure S2D).



Supplementary material 2: pH shifts of four treeline soil types after 3 (A, B), 7 (C) and 21 (D) days of adding different test substrates as 1ml solution in water to 5 g to subsets of each soil (A-C) and pH in all replicate soils of the incubation under the highest C:N (7:1) addition (D). pH was measured in water. Average pH shifts were -0.16 ± 0.11 . Abbreviations: Gl is Puna grassland in Peru and tundra heath in Sweden, org: organic soil horizon, min: mineral soil horizon, ALA: alanine, G: glucose, SC: substrate-C, FWT: soil fresh weight