

1 **Vertical redistribution of soil organic carbon pools after twenty years of nitrogen addition in two**
2 **temperate coniferous forests**

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1 **Author contributions** S.J.F contributed to study design, performed research, analyzed data and wrote
2 the manuscript; V.W. and S.M. performed research; K.M.K, E.D.-P., F.H. and W.W. co-wrote the
3 manuscript; P.S. and P.G. maintained the N addition experiments, provided data and co-wrote the
4 manuscript; M.T. conceived the study and performed research; M.H.G conceived the study and
5 co-wrote the manuscript; S.Z-B conceived the study, co-wrote the manuscript, was the first supervisor
6 of students and led the project.

17 **Supplementary Material**

18 **Appendix S1:** Physicochemical soil properties – Supplementary Tables (4)

19 **Appendix S2:** Tree growth and N accumulation – Supplementary Table (1)

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27 Supplementary Text and Supplementary Figure (1)

28 Appendix S1: Physicochemical soil properties

29 **Table S1.1** Gravimetric water content (GWC), soil texture, soil pH, carbonate concentration (CaCO₃), cation exchange capacity (CEC_{eff}),
 30 base saturation (BS_{eff}), ammonium-N (NH₄⁺-N), nitrate-N (NO₃⁻-N), extractable organic carbon (EOC), extractable organic nitrogen (EON)
 31 and EOC:EON (mass ratio) in genetic soil horizons at Alptal. Lower-case letters within columns originate from pairwise comparison of
 32 horizon means. Means with no letter in common are significantly different (Tukey's HSD; $\alpha = 0.05$). Bold and bold-italic values indicate
 33 significant ($P < 0.05$) and marginally significant ($P < 0.1$) post-hoc differences between treatments within a horizon, respectively. Note that
 34 post-hoc differences were found for NH₄⁺-N despite a non-significant interaction. Means ($\pm$ SE) were derived from 4-13 samples per
 35 horizon/treatment combination.

	GWC	Sand	Silt	Clay	pH	CaCO ₃	CEC _{eff} (mmol _c kg ⁻¹ soil)	BS _{eff}	NH ₄ ⁺ -N	NO ₃ ⁻ -N	EOC	EON	EOC:EON
	(%)	(%)	(%)	(%)		(%)		(%)	(μ g g ⁻¹ soil)	(μ g g ⁻¹ soil)	(μ g g ⁻¹ soil)	(μ g g ⁻¹ soil)	(mass ratio)
<i>Oi</i>	a	n.a.	n.a.	n.a.	ab	n.a.	n.a.	n.a.	a	a	a	a	ab
Control	66.7 $\pm$ 2.4				6.0 $\pm$ 0.1				410.8 $\pm$ 49.7	1.1 $\pm$ 0.4	5614.9 $\pm$ 1032.6	687.8 $\pm$ 63.9	7.6 $\pm$ 0.9
+ Nitrogen	66.9 $\pm$ 4.0				5.7 $\pm$ 0.2				465.2 $\pm$ 71.0	9.7 $\pm$ 3.8	3376.0 $\pm$ 449.7	587.5 $\pm$ 54.2	6.0 $\pm$ 0.7
<i>Oe</i>	ab	n.a.	n.a.	n.a.	ac	n.a.	n.a.	n.a.	a	a	b	b	ac
Control	74.5 $\pm$ 2.6				5.6 $\pm$ 0.3				468.1 $\pm$ 158.6	1.9 $\pm$ 0.8	2539.3 $\pm$ 377.3	465.7 $\pm$ 61.9	5.7 $\pm$ 0.6
+ Nitrogen	74.9 $\pm$ 2.9				5.2 $\pm$ 0.2				730.2 $\pm$ 113.7	5.1 $\pm$ 2.8	1568.1 $\pm$ 127.2	476.2 $\pm$ 59.1	3.6 $\pm$ 0.4
<i>Oa</i>	b	n.a.	n.a.	n.a.	c	n.a.	n.a.	n.a.	b	b	b	b	c
Control	78.7 $\pm$ 1.8				5.5 $\pm$ 0.3				172.6 $\pm$ 14.7	n.d.	1669.4 $\pm$ 169.0	368.3 $\pm$ 22.4	4.5 $\pm$ 0.3
+ Nitrogen	75.0 $\pm$ 3.4				4.6 $\pm$ 0.1				239.7 $\pm$ 52.3	2.6 $\pm$ 2.0	1207.4 $\pm$ 88.3	417.9 $\pm$ 50.7	3.0 $\pm$ 0.3
<i>Ah</i>	a	a	a		c				c	a	c	c	b
Control	69.3 $\pm$ 2.6	2.6 $\pm$ 1.3	38.1 $\pm$ 8.8	59.3 $\pm$ 10.0	5.3 $\pm$ 0.2	0.5 $\pm$ 0.3	559.6 $\pm$ 136.9	93.6 $\pm$ 5.2	23.5 $\pm$ 7.0	9.2 $\pm$ 3.2	463.6 $\pm$ 51.8	73.7 $\pm$ 10.9	6.7 $\pm$ 0.4
+ Nitrogen	64.3 $\pm$ 2.6	1.6 $\pm$ 0.4	35.5 $\pm$ 0.9	62.9 $\pm$ 1.1	5.1 $\pm$ 0.2	0.3 $\pm$ 0.1	292.4 $\pm$ 115.5	89.8 $\pm$ 6.0	37.2 $\pm$ 9.7	9.4 $\pm$ 4.8	412.0 $\pm$ 31.2	78.1 $\pm$ 8.0	5.6 $\pm$ 0.4
<i>Bl</i>	c	a	a		b				d	a	d	d	ab
Control	47.8 $\pm$ 2.0	2.3 $\pm$ 0.9	39.2 $\pm$ 2.9	58.5 $\pm$ 3.7	6.2 $\pm$ 0.2	0.3 $\pm$ 0.1	265.5 $\pm$ 36.9	97.4 $\pm$ 1.8	4.5 $\pm$ 0.8	4.3 $\pm$ 1.4	117.4 $\pm$ 17.7	20.9 $\pm$ 2.4	5.5 $\pm$ 0.4
+ Nitrogen	48.0 $\pm$ 2.7	2.0 $\pm$ 0.5	38.1 $\pm$ 2.8	60.0 $\pm$ 3.0	5.8 $\pm$ 0.2	0.3 $\pm$ 0.1	196.2 $\pm$ 57.9	69.9 $\pm$ 13.1	5.9 $\pm$ 2.1	2.6 $\pm$ 1.3	136.6 $\pm$ 14.1	22.3 $\pm$ 2.3	6.5 $\pm$ 0.7
<i>Blr^a</i>	c	a	a		d				d	ab	e	e	b
Control	43.6 $\pm$ 1.3	2.0 $\pm$ 0.6	39.0 $\pm$ 0.9	59.0 $\pm$ 0.9	6.6 $\pm$ 0.2	0.6 $\pm$ 0.4	349.4 $\pm$ 8.9	99.9 $\pm$ 0.1	4.1 $\pm$ 0.7	0.6 $\pm$ 0.2	74.3 $\pm$ 11.5	11.9 $\pm$ 1.1	6.5 $\pm$ 1.1
+ Nitrogen	43.1 $\pm$ 1.3	1.6 $\pm$ 0.4	41.0 $\pm$ 1.8	57.4 $\pm$ 1.9	6.6 $\pm$ 0.2	0.7 $\pm$ 0.4	242.6 $\pm$ 39.6	99.3 $\pm$ 0.4	2.6 $\pm$ 0.5	1.0 $\pm$ 0.4	62.6 $\pm$ 13.2	3.7 $\pm$ 0.9	28.9 $\pm$ 14.0
Sign. effects	Horizon				+N, Horizon		+N		Horizon	Horizon, +N x Horizon	+N, Horizon	+N ^b , Horizon, +N x Horizon	Horizon, +N x Horizon

n.a. = not analyzed.

^a Blr horizons were sampled to an average depth of 30.0 $\pm$ 2.1 cm and 26.3 $\pm$ 1.0 cm from top of mineral soil in control and +N treatment plots, respectively.

^b Marginally significant main effect ($P=0.089$).

37 **Table S1.2** Exchangeable cations in mineral soil horizons at Alptal. Lower-case letters within columns originate from pairwise comparison
 38 of horizon means. Means with no letter in common are significantly different (Tukey's HSD; $\alpha = 0.05$). Means ($\pm$ SE) were derived from 4
 39 samples per horizon/treatment combination.

	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Base cations (mmol _c kg ⁻¹ soil)	Al ³⁺	Mn ²⁺	Fe ³⁺	Acidic cations
<i>Ah</i>			<i>a</i>	<i>a</i>		<i>ab</i>			<i>a</i>
Control	0.54 ± 0.05	3.34 ± 0.85	17.54 ± 2.97	520.63 ± 145.94	542.05 ± 147.46	13.45 ± 9.73	1.33 ± 0.43	2.80 ± 1.68	17.57 ± 11.56
+ Nitrogen	0.45 ± 0.06	2.73 ± 0.44	13.21 ± 2.91	256.91 ± 118.57	273.31 ± 121.16	17.97 ± 10.89	0.93 ± 0.54	0.17 ± 0.14	19.07 ± 10.77
<i>Bl</i>			<i>a</i>	<i>a</i>		<i>a</i>			<i>a</i>
Control	0.46 ± 0.03	2.46 ± 0.17	10.87 ± 0.50	246.04 ± 38.98	259.82 ± 39.37	5.07 ± 3.77	0.34 ± 0.10	0.28 ± 0.25	5.68 ± 3.92
+ Nitrogen	0.55 ± 0.12	2.68 ± 0.37	10.05 ± 1.42	140.73 ± 70.79	154.01 ± 71.98	40.53 ± 17.51	0.49 ± 0.16	1.22 ± 0.68	42.24 ± 18.11
<i>Blr^e</i>			<i>a</i>	<i>a</i>		<i>b</i>			<i>b</i>
Control	0.49 ± 0.05	2.39 ± 0.13	9.66 ± 2.16	336.61 ± 6.58	349.14 ± 8.64	0.02 ± 0.02	0.28 ± 0.21	0.00 ± 0.00	0.29 ± 0.23
+ Nitrogen	0.43 ± 0.05	2.24 ± 0.15	11.16 ± 0.61	227.41 ± 40.00	241.24 ± 40.10	0.21 ± 0.12	0.56 ± 0.22	0.58 ± 0.35	1.34 ± 0.69
Sign. effects			<i>Horizon^b</i>	<i>+N, Horizon^c</i>	<i>+N, Horizon^d</i>	<i>Horizon</i>			<i>Horizon</i>

^aBlr horizons were sampled to an average depth of 30.0±2.1 cm and 26.3±1.0 cm from top of mineral soil in control and N addition plots, respectively.
 Marginally significant main effects (^bP=0.086,^cP=0.068,^dP=0.070).

Table S1.3 Gravimetric water content (GWC), soil texture, soil pH, carbonate concentration (CaCO₃), cation exchange capacity (CEC_{eff}), base saturation (BS_{eff}), ammonium-N (NH₄⁺-N), nitrate-N (NO₃⁻-N), extractable organic carbon (EOC), extractable organic nitrogen (EON) and EOC:EON (mass ratio) in genetic soil horizons at Klosterhede. Lower-case letters within columns originate from pairwise comparison of horizon means. Means with no letter in common are significantly different (Tukey's HSD; $\alpha = 0.05$). Bold and bold-italic values indicate significant ($P < 0.05$) and marginally significant ($P < 0.1$) post-hoc differences between treatments within a horizon, respectively. Note that post-hoc differences were found for soil pH despite a non-significant interaction. Means ($\pm$ SE) were derived from 3-12 samples per horizon/treatment combination.

	GWC (%)	Sand (%)	Silt (%)	Clay (%)	pH	CEC _{eff} (mmol _c kg ⁻¹ soil)	BS _{eff} (%)	NH ₄ ⁺ -N (μ g g ⁻¹ soil)	NO ₃ ⁻ -N (μ g g ⁻¹ soil)	EOC (μ g g ⁻¹ soil)	EON (μ g g soil ⁻¹)	EOC:EON (mass ratio)
Oe	<i>a</i>	n.a.	n.a.	n.a.	<i>a</i>	n.a.	n.a.	<i>a</i> [†]	<i>a</i> [†]	<i>a</i>	<i>a</i> [†]	<i>a</i>
Control	69.0 $\pm$ 0.5				4.4 $\pm$ 0.0			230.3 $\pm$ 18.4	n.d.	1221.3 $\pm$ 85.4	263.2 $\pm$ 18.9	4.7 $\pm$ 0.3
+ Nitrogen	67.4 $\pm$ 0.9				4.5 $\pm$ 0.0			425.4 $\pm$ 25.0	1.4 $\pm$ 0.6	1236.6 $\pm$ 101.0	456.8 $\pm$ 27.2	2.7 $\pm$ 0.2
Oa	<i>a</i>	n.a.	n.a.	n.a.	<i>b</i>	n.a.	n.a.	<i>b</i> [†]	<i>b</i> [†]	<i>b</i>	<i>b</i> [†]	<i>a</i>
Control	64.8 $\pm$ 1.0				4.3 $\pm$ 0.0			117.4 $\pm$ 9.6	n.d.	686.1 $\pm$ 44.9	149.1 $\pm$ 9.7	4.7 $\pm$ 0.3
+ Nitrogen	62.1 $\pm$ 2.6				4.2 $\pm$ 0.0			172.8 $\pm$ 14.3	4.1 $\pm$ 1.0	698.0 $\pm$ 48.2	198.9 $\pm$ 12.1	3.7 $\pm$ 0.3
AE	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i> [†]	<i>a</i> [†]	<i>c</i>	<i>ab</i> [‡]	<i>b</i>
Control	15.6 $\pm$ 0.7	77.7 $\pm$ 3.6	17.0 $\pm$ 2.9	5.3 $\pm$ 0.7	4.5 $\pm$ 0.0	5.3 $\pm$ 1.0	58.3 $\pm$ 8.6	3.6 $\pm$ 0.5	0.1 $\pm$ 0.0	84.8 $\pm$ 6.8	10.2 $\pm$ 0.6	8.4 $\pm$ 0.6
+ Nitrogen	17.2 $\pm$ 1.1	73.4 $\pm$ 1.5	19.7 $\pm$ 0.5	6.8 $\pm$ 1.3	4.5 $\pm$ 0.1	6.2 $\pm$ 1.7	61.6 $\pm$ 3.9	6.9 $\pm$ 1.0	0.4 $\pm$ 0.1	90.1 $\pm$ 7.1	15.1 $\pm$ 1.2	6.2 $\pm$ 0.5
E	<i>c</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>ac</i>	<i>a</i>	<i>b</i>	<i>b</i> [†]	<i>b</i> [†]	<i>c</i>	<i>a</i> [†]	<i>b</i>
Control	12.0 $\pm$ 0.5	77.7 $\pm$ 0.5	15.5 $\pm$ 0.7	6.8 $\pm$ 0.2	4.6 $\pm$ 0.0	5.1 $\pm$ 1.0	33.4 $\pm$ 4.0	2.5 $\pm$ 0.5	n.d.	77.4 $\pm$ 11.5	10.3 $\pm$ 1.4	8.0 $\pm$ 1.1
+ Nitrogen	12.4 $\pm$ 0.5	78.3 $\pm$ 1.2	15.3 $\pm$ 1.4	6.3 $\pm$ 0.6	4.5 $\pm$ 0.1	4.4 $\pm$ 0.6	29.7 $\pm$ 6.6	4.5 $\pm$ 0.5	0.2 $\pm$ 0.1	83.6 $\pm$ 8.1	13.1 $\pm$ 1.1	6.7 $\pm$ 0.6
Bh	<i>d</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>b</i>	<i>b</i> [†]	<i>b</i> [†]	<i>d</i>	<i>c</i> [‡]	<i>c</i>
Control	25.3 $\pm$ 1.0	62.3 $\pm$ 1.9	21.1 $\pm$ 2.9	16.7 $\pm$ 1.4	4.5 $\pm$ 0.1	12.2 $\pm$ 1.0	25.8 $\pm$ 1.0	1.7 $\pm$ 0.4	n.d.	379.3 $\pm$ 25.3	15.1 $\pm$ 0.8	25.6 $\pm$ 1.6
+ Nitrogen	24.9 $\pm$ 0.7	64.2 $\pm$ 1.3	18.1 $\pm$ 1.0	17.7 $\pm$ 1.6	4.6 $\pm$ 0.0	10.5 $\pm$ 1.2	23.7 $\pm$ 2.4	5.4 $\pm$ 1.1	0.2 $\pm$ 0.1	353.7 $\pm$ 20.7	17.0 $\pm$ 1.6	21.9 $\pm$ 1.5
BS^a	<i>e</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>c</i>	<i>b</i>	<i>b</i> [†]	<i>a</i> [†]	<i>e</i>	<i>bc</i> [‡]	<i>c</i>
Control	20.2 $\pm$ 0.7	66.8 $\pm$ 2.1	21.9 $\pm$ 2.0	11.3 $\pm$ 0.7	4.6 $\pm$ 0.0	2.5 $\pm$ 0.1	28.6 $\pm$ 2.2	2.6 $\pm$ 0.4	0.2 $\pm$ 0.1	291.0 $\pm$ 27.6	13.2 $\pm$ 0.7	21.7 $\pm$ 1.3
+ Nitrogen	17.9 $\pm$ 0.4	71.5 $\pm$ 2.2	18.7 $\pm$ 1.5	9.8 $\pm$ 0.7	4.7 $\pm$ 0.1	2.6 $\pm$ 0.2	24.8 $\pm$ 1.8	3.4 $\pm$ 0.4	0.4 $\pm$ 0.1	245.0 $\pm$ 20.3	13.8 $\pm$ 0.6	17.7 $\pm$ 1.2
Sign. effects	Horizon	Horizon	Horizon ^b	Horizon	Horizon	Horizon	Horizon	Org: +N, Horizon Min: +N, Horizon	Org: +N, Horizon, +N x Horizon Min: +N, Horizon	Horizon	Org: +N, Horizon Min: +N, Horizon, +N x Horizon ^c	+N, Horizon, +N x Horizon

n.d. = not detected; n.a. = not analyzed.

^a Bs horizons were sampled to an average depth of 31.6 $\pm$ 1.3 cm and 29.7 $\pm$ 0.8 cm from top of mineral soil in control and +N treatment plots, respectively.

Marginally significant main effect and treatment x horizon interactions (*P=0.071, [†]P=0.097, [‡]P=0.099).

[†] Lower-case letters indicate significant post-hoc differences between organic horizons.

[‡] Lower-case letters indicate significant post-hoc differences between mineral horizons.

49 **Table S1.4** Exchangeable cations in mineral soil horizons at Klosterhede. Lower-case letters within columns originate from pairwise
50 comparison of horizon means. Means with no letter in common are significantly different (Tukey's HSD; $\alpha = 0.05$). Means ($\pm$ SE) were
51 derived from 4 samples per horizon/treatment combination.

	Na ⁺	K ⁺	Mg ²⁺	Ca ²⁺	Base cations (mmol _c kg ⁻¹ soil)	Al ³⁺	Mn ²⁺	Fe ³⁺	Acidic cations
<i>AE</i>	<i>ab</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>		<i>a</i>	<i>a</i>
Control	1.00 $\pm$ 0.38	0.29 $\pm$ 0.05	1.83 $\pm$ 0.61	0.19 $\pm$ 0.09	3.31 $\pm$ 1.12	0.59 $\pm$ 0.05	n.d.	1.40 $\pm$ 0.24	1.99 $\pm$ 0.24
+ Nitrogen	1.12 $\pm$ 0.37	0.33 $\pm$ 0.07	2.26 $\pm$ 0.77	0.23 $\pm$ 0.09	3.94 $\pm$ 1.30	0.81 $\pm$ 0.17	n.d.	1.42 $\pm$ 0.28	2.23 $\pm$ 0.44
<i>E</i>	<i>ac</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>b</i>		<i>a</i>	<i>b</i>
Control	0.58 $\pm$ 0.10	0.19 $\pm$ 0.02	0.85 $\pm$ 0.20	0.11 $\pm$ 0.03	1.73 $\pm$ 0.35	1.94 $\pm$ 0.32	n.d.	1.48 $\pm$ 0.34	3.42 $\pm$ 0.66
+ Nitrogen	0.48 $\pm$ 0.09	0.21 $\pm$ 0.02	0.46 $\pm$ 0.10	0.09 $\pm$ 0.02	1.24 $\pm$ 0.22	2.05 $\pm$ 0.42	n.d.	1.16 $\pm$ 0.24	3.20 $\pm$ 0.66
<i>Bh</i>	<i>b</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>c</i>		<i>b</i>	<i>c</i>
Control	1.28 $\pm$ 0.16	0.36 $\pm$ 0.01	1.40 $\pm$ 0.24	0.13 $\pm$ 0.02	3.17 $\pm$ 0.37	6.56 $\pm$ 0.31	n.d.	2.48 $\pm$ 0.37	9.04 $\pm$ 0.66
+ Nitrogen	0.92 $\pm$ 0.11	0.34 $\pm$ 0.03	1.03 $\pm$ 0.21	0.15 $\pm$ 0.03	2.44 $\pm$ 0.30	5.91 $\pm$ 0.65	n.d.	2.10 $\pm$ 0.49	8.01 $\pm$ 0.99
<i>Bs</i> ^a	<i>c</i>	<i>b</i>	<i>c</i>	<i>b</i>	<i>c</i>	<i>b</i>		<i>c</i>	<i>a</i>
Control	0.45 $\pm$ 0.08	0.12 $\pm$ 0.02	0.13 $\pm$ 0.01	0.03 $\pm$ 0.02	0.72 $\pm$ 0.07	1.43 $\pm$ 0.06	n.d.	0.35 $\pm$ 0.04	1.78 $\pm$ 0.06
+ Nitrogen	0.36 $\pm$ 0.06	0.12 $\pm$ 0.02	0.14 $\pm$ 0.03	0.03 $\pm$ 0.00	0.65 $\pm$ 0.09	1.56 $\pm$ 0.11	n.d.	0.39 $\pm$ 0.06	1.95 $\pm$ 0.17
Sign. effects	Horizon	Horizon	Horizon	Horizon		Horizon		Horizon	

^aBs horizons were sampled to an average depth of 31.6 $\pm$ 1.3 cm and 29.7 $\pm$ 0.8 cm from top of mineral soil in control and +N treatment plots, respectively.

53 **Appendix S2: Tree growth and N accumulation**

54 **Table S2.1** Linear regression parameters of selected aboveground and belowground responses to N
 55 addition as depicted in Figure 3 of the main text.

56

Equation	df	P(Slope)	RSE	R ²	Adj. R ²
<i>Panel (a) – Alptal</i>					
$BAI = 1.15 \times Year - 2219.53$	12	<0.001	3.16	0.71	0.69
$Needle\ biomass = 1.16 \times Year - 2199.22$	13	0.048	8.85	0.27	0.21
<i>Panel (b) – Klosterhede</i>					
$BAI = -0.84 \times Year + 1765.98$	19	0.262	22.29	0.07	0.02
† $BAI = -1.59 \times Year + 3231.02$	18	0.023	18.14	0.26	0.21
<i>Panel (c) – Alptal</i>					
$Needle\ Mg: N = -1.61 \times Year + 3300.07$	9	0.015	5.61	0.50	0.45
$Litter\ N = 1.67 \times Year - 3237.4$	12	0.002	6.49	0.56	0.52
<i>Panel (d) – Klosterhede</i>					
¶ $Needle\ N\ conc. = 1.08 \times Year - 2034.90$	4	0.041	5.40	0.69	0.61
¶ $Needle\ Mg: N = -1.23 \times Year + 2536.86$	4	0.111	9.08	0.51	0.39
$Litter\ N = 1.86 \times Year - 3600.0$	3	0.004	3.39	0.96	0.94

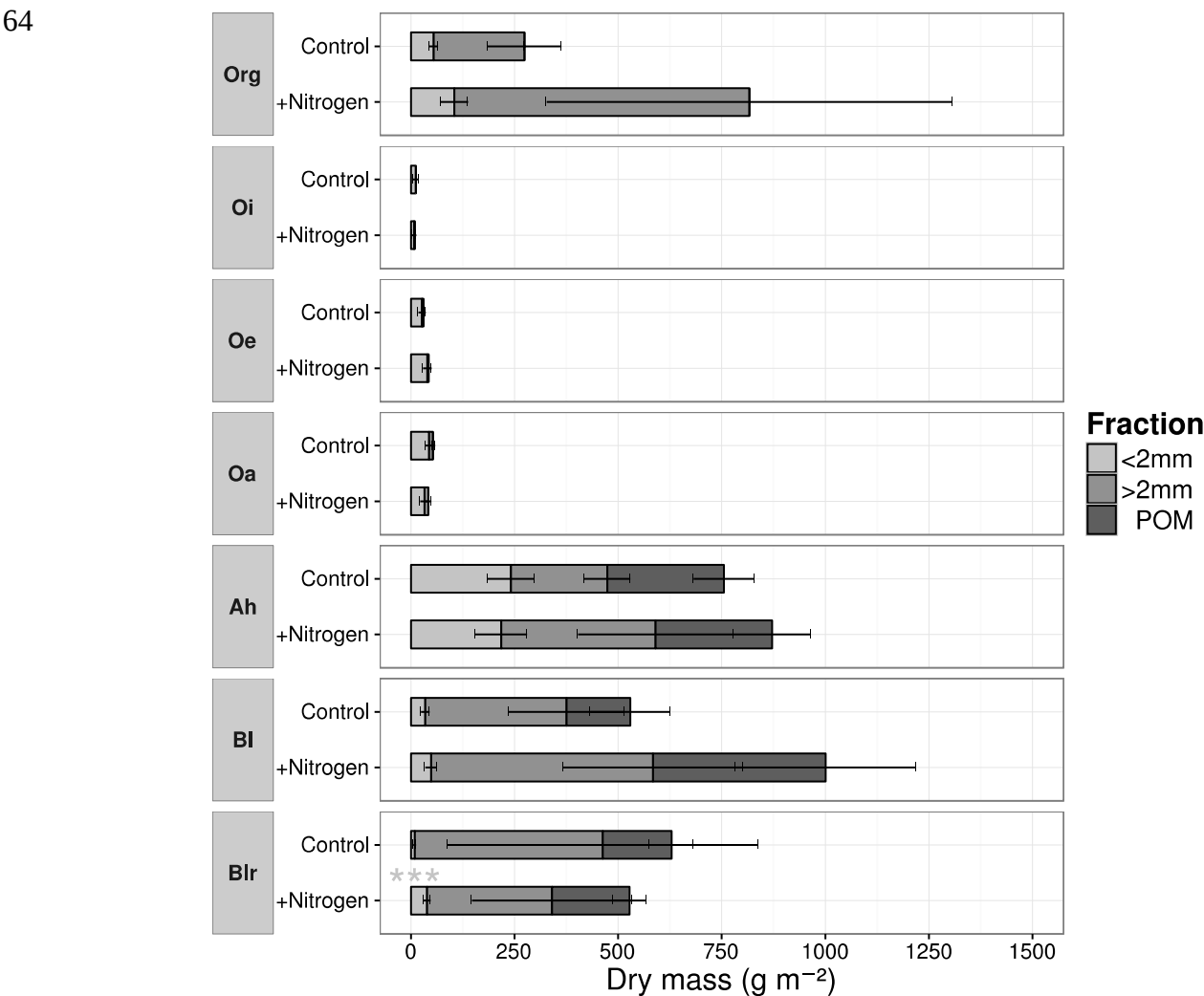
RSE stands for residual standard error, BAI stands for basal area increment.

† Linear regression excluding data from 1992.

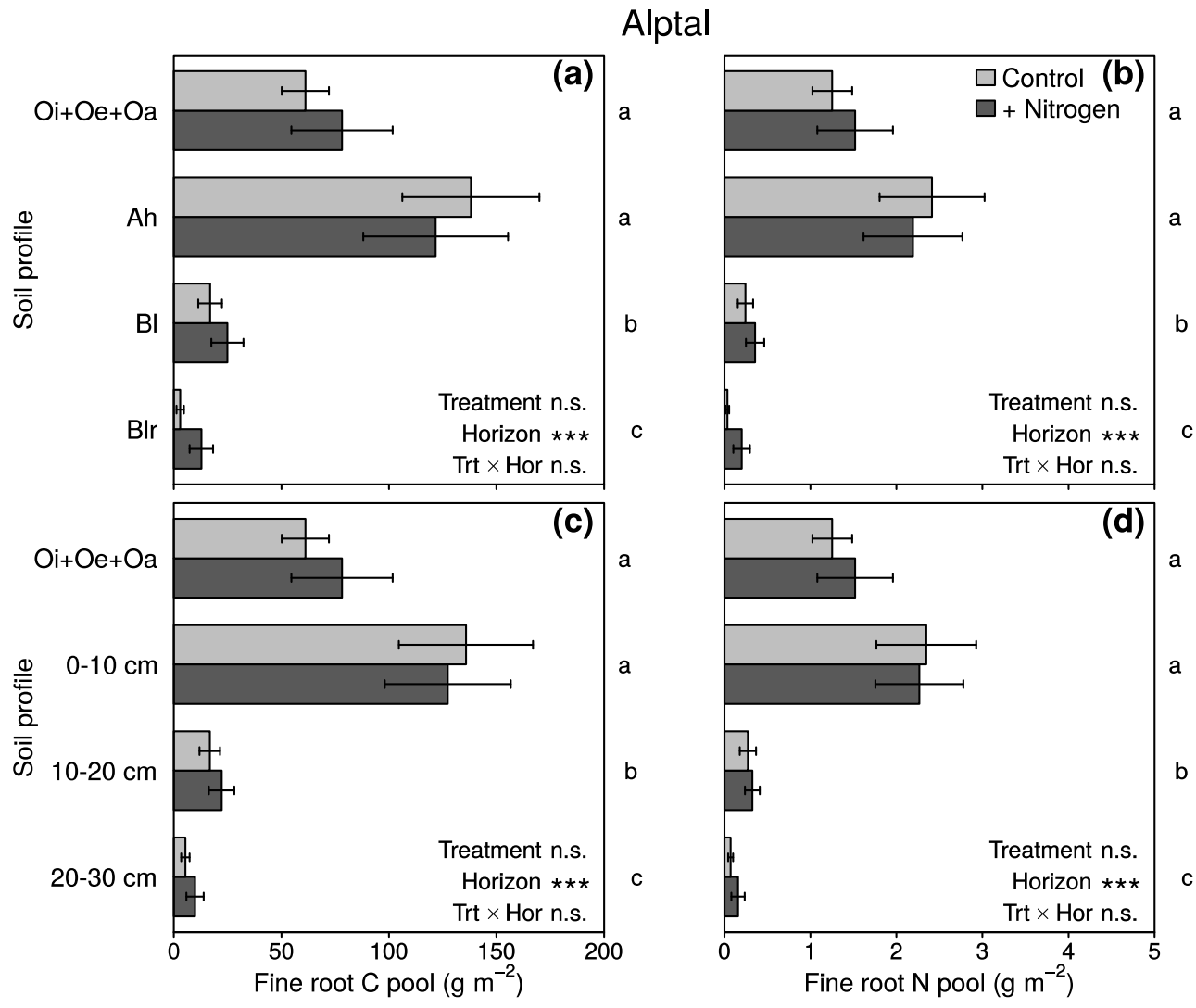
¶ Linear regression excluding data from 1999 (no N was applied in 1998-1999).

57 **Appendix S3: Fine roots**

58 **Figure S3.1** Fine root mass (<2 mm diameter), coarse root mass (>2 mm diameter) and particulate
59 organic matter mass (POM) in genetic soil horizons at Alptal. Roots that could not be assigned to a
60 specific organic horizon were measured separately ('Org'). Particulate organic matter was not
61 determined for these horizons ('Org'). Symbols between bars indicate significant post-hoc differences
62 for a given fraction between treatments within a given horizon ($\blacksquare P < 0.1$, $*P < 0.05$, $**P < 0.01$,
63 $***P < 0.001$). Means ($\pm$ SE) were derived from 6-13 samples per horizon/treatment combination.



65 **Figure S3.2** Fine root C pools (a,c) and fine root N pools (b,d) in genetic soil horizons (upper panels)
 66 and depth increments (lower panels) at Alptal. Lower-case letters right to each plate originate from
 67 pairwise comparison of horizon/increment means. Means with no letter in common are significantly
 68 different (Tukey's HSD; $\alpha = 0.05$). Means ($\pm$ SE) were derived from 12 samples per horizon/treatment
 69 combination.



71 **Figure S3.3** Fine root mass (<2mm diameter), coarse root mass (>2mm diameter) and particulate
 72 organic matter mass (POM) in genetic soil horizons at Klosterhede. Roots that could not be assigned to
 73 a specific organic horizon were measured separately ('Org'). Particulate organic matter was not
 74 determined for these horizons ('Org'). Symbols between bars indicate significant post-hoc differences
 75 for a given fraction between treatments within a given horizon ($\blacksquare P < 0.1$, $*P < 0.05$, $**P < 0.01$,
 76 $***P < 0.001$). The N addition main effects for fine root mass and coarse root mass are significant at
 77 $P = 0.016$ and $P = 0.060$, respectively. Means ($\pm$ SE) were derived from 9-12 samples per
 78 horizon/treatment combination.

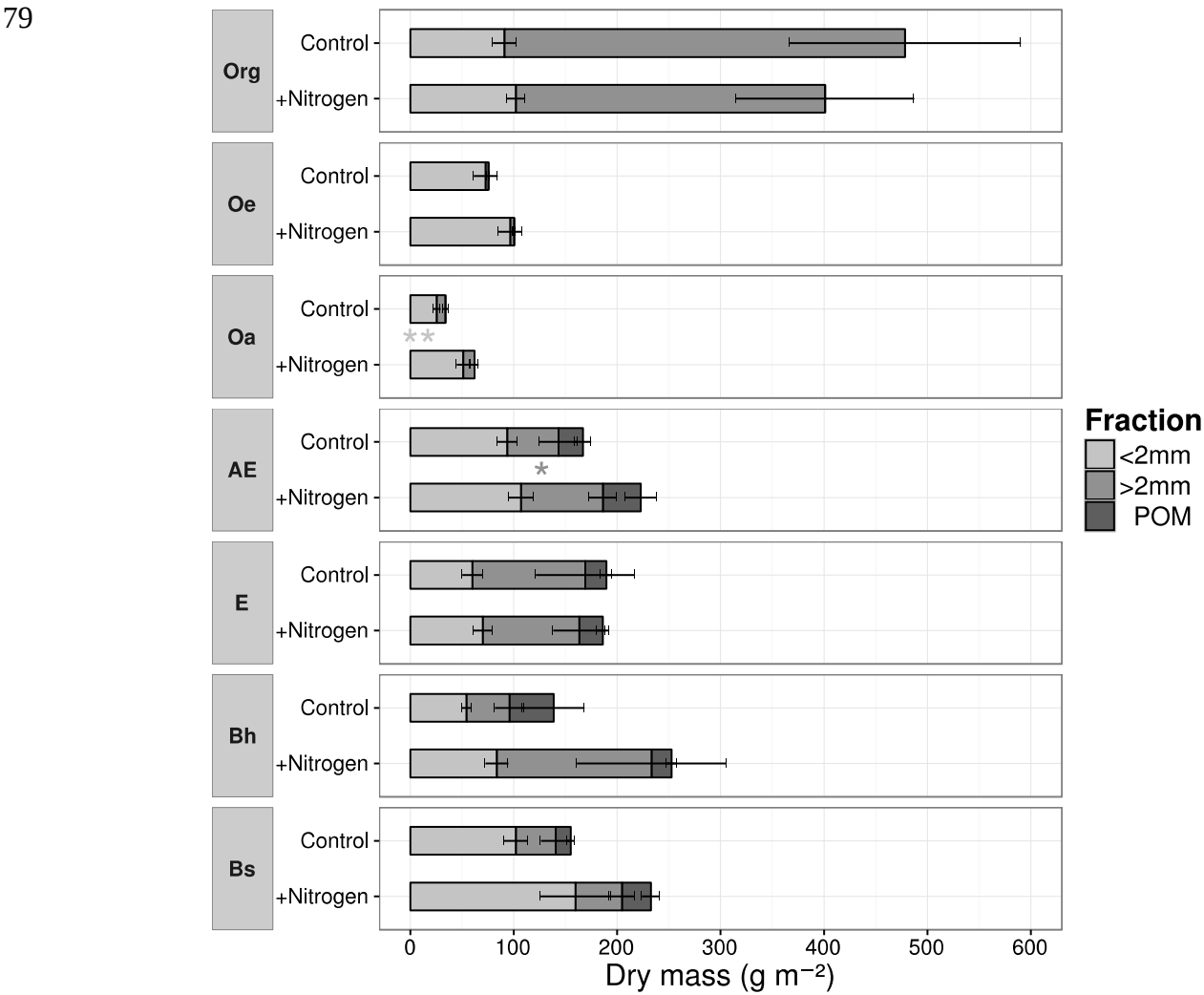


Table S3.1 Fine root C (FRC) concentrations, fine root N (FRN) concentrations and FRC:FRN (mass ratio) in genetic soil horizons at Alptal. Roots that could not be assigned to a specific organic horizon were measured separately ('Org'). Lower-case letters within columns originate from pairwise comparison of horizon means. Means with no letter in common are significantly different (Tukey's HSD; $\alpha = 0.05$). Bold and bold-italic values indicate significant ($P < 0.05$) and marginally significant ($P < 0.1$) post-hoc differences between treatments within a horizon, respectively. Note that post-hoc differences were found for FRC concentrations in the Bl horizon, as well as for FRN concentrations and FRC:FRN in the Ah horizon, despite non-significant interactions. Means ($\pm$ SE) were derived from 3-13 samples per horizon/treatment combination.

	Fine root C (mass%)	Fine root N (mass%)	FRC:FRN (mass ratio)	n
<i>Org</i>				
Control	53.60 $\pm$ 0.57	0.95 $\pm$ 0.06	59.09 $\pm$ 4.05	11
+ Nitrogen	54.26 $\pm$ 0.37	1.01 $\pm$ 0.08	57.16 $\pm$ 4.56	11
<i>Oi</i>		<i>a</i>	<i>a</i>	
Control	49.87 $\pm$ 0.33	1.40 $\pm$ 0.11	36.60 $\pm$ 3.43	5
+ Nitrogen	50.74 $\pm$ 1.52	1.50 $\pm$ 0.17	35.54 $\pm$ 4.87	4
<i>Oe</i>		<i>a</i>	<i>a</i>	
Control	51.01 $\pm$ 0.55	1.34 $\pm$ 0.13	40.67 $\pm$ 4.29	7
+ Nitrogen	51.89 $\pm$ 0.27	1.36 $\pm$ 0.06	38.83 $\pm$ 2.34	8
<i>Oa</i>		<i>a</i>	<i>a</i>	
Control	50.80 $\pm$ 0.48	1.14 $\pm$ 0.06	45.84 $\pm$ 2.24	11
+ Nitrogen	52.38 $\pm$ 0.30	1.14 $\pm$ 0.07	46.92 $\pm$ 2.83	6
<i>Ah</i>		<i>b</i>	<i>b</i>	
Control	51.89 $\pm$ 0.71	0.83 $\pm$ 0.05	65.30 $\pm$ 4.08	13
+ Nitrogen	51.20 $\pm$ 0.65	1.04 $\pm$ 0.07	51.55 $\pm$ 3.22	13
<i>Bl</i>		<i>c</i>	<i>c</i>	
Control	50.07 $\pm$ 0.71	0.70 $\pm$ 0.05	74.76 $\pm$ 6.79	9
+ Nitrogen	51.64 $\pm$ 0.41	0.71 $\pm$ 0.07	84.61 $\pm$ 17.07	8
<i>Blr^a</i>		<i>bc</i>	<i>bc</i>	
Control	52.32 $\pm$ 0.47	0.68 $\pm$ 0.14	85.18 $\pm$ 19.06	3
+ Nitrogen	51.02 $\pm$ 0.74	0.76 $\pm$ 0.10	70.80 $\pm$ 6.64	5
Sign. effects		Horizon	Horizon	

^a Blr horizons were sampled to an average depth of 30.0 $\pm$ 2.1 cm and 26.3 $\pm$ 1.0 cm from top of mineral soil in control and +N treatment plots, respectively.

Table S3.2 Fine root C (FRC) concentrations, fine root N (FRN) concentrations and FRC:FRN (mass ratio) in genetic soil horizons at Klosterhede. Roots that could not be assigned to a specific organic horizon have been measured separately ('Org'). Lower-case letters within columns originate from pairwise comparison of horizon means. Means with no letter in common are significantly different (Tukey's HSD; $\alpha = 0.05$). Bold values indicate significant N addition effects for 'Org' roots ($P < 0.05$). Bold-italic values indicate marginally significant ($P < 0.1$) post-hoc differences between treatments within a horizon. Note that post-hoc differences were found for FRC concentrations in the Bs horizon despite a non-significant interaction. Means ($\pm$ SE) were derived from 9-12 samples.

	Fine root C (mass%)	Fine root N (mass%)	FRC:FRN (mass ratio)	n
<i>Org</i>				
Control	47.62 $\pm$ 0.09	0.71 $\pm$ 0.02	67.57 $\pm$ 1.90	12
+ Nitrogen	47.70 $\pm$ 0.09	1.04 $\pm$ 0.02	46.15 $\pm$ 0.88	12
<i>Oe</i>	<i>a</i>	<i>a</i>	<i>a</i>	
Control	47.45 $\pm$ 0.12	1.01 $\pm$ 0.02	47.07 $\pm$ 1.04	12
+ Nitrogen	47.39 $\pm$ 0.08	1.35 $\pm$ 0.02	35.31 $\pm$ 0.50	12
<i>Oa</i>	<i>b</i>	<i>b</i>	<i>b</i>	
Control	46.70 $\pm$ 0.12	0.91 $\pm$ 0.02	51.63 $\pm$ 1.41	12
+ Nitrogen	46.59 $\pm$ 0.11	1.09 $\pm$ 0.03	43.27 $\pm$ 1.33	10
<i>AE</i>	<i>b</i>	<i>b</i>	<i>bc</i>	
Control	46.55 $\pm$ 0.21	0.81 $\pm$ 0.02	57.89 $\pm$ 1.93	12
+ Nitrogen	46.24 $\pm$ 0.22	1.11 $\pm$ 0.04	42.23 $\pm$ 1.48	12
<i>E</i>	<i>c</i>	<i>c</i>	<i>cd</i>	
Control	43.52 $\pm$ 1.08	0.71 $\pm$ 0.03	62.48 $\pm$ 3.29	11
+ Nitrogen	43.56 $\pm$ 0.87	0.99 $\pm$ 0.04	44.87 $\pm$ 2.12	12
<i>Bh</i>	<i>d</i>	<i>c</i>	<i>d</i>	
Control	42.79 $\pm$ 0.45	0.69 $\pm$ 0.02	62.11 $\pm$ 1.93	10
+ Nitrogen	42.49 $\pm$ 0.57	0.90 $\pm$ 0.04	48.29 $\pm$ 2.67	9
<i>Bs^a</i>	<i>d</i>	<i>d</i>	<i>e</i>	
Control	42.80 $\pm$ 0.29	0.54 $\pm$ 0.02	80.12 $\pm$ 3.18	9
+ Nitrogen	40.09 $\pm$ 1.28	0.71 $\pm$ 0.04	57.53 $\pm$ 1.67	11
Sign. effects	Horizon	+N, Horizon, +N x Horizon ^b	+N, Horizon	

^aBs horizons were sampled to an average depth of 31.6 $\pm$ 1.3 cm and 29.7 $\pm$ 0.8 cm from top of mineral soil in control and +N treatment plots, respectively.

^bMarginally significant interaction ($P=0.087$).

99 **Appendix S4:** Soil organic C and soil total N pools

100 For estimation of SOC and STN pools (Equation 1 in the main text), we calculated bulk density ($\rho_{B,i}$) as

$$\rho_{B,i} = \frac{M_i}{V_i} \quad (S1)$$

101 where M_i is dry mass (kg) and V_i is the volume (m^3) of root-free (for organic horizons) or 2mm-sieved
102 (for mineral horizons) soil material, respectively. Soil volume V_i (m^3) was calculated for horizon i by
103 correcting the nominal sampling volume $V_{N,i}$ for volume of roots and stones as

$$V_i = V_{N,i} - V_{R,i} - V_{S,i} = (A_{N,i} \times d_i) - FM_{R,i} - \left(\frac{M_{S,i}}{2.65} \right) \quad (S2)$$

104 where $V_{N,i}$ is the nominal sampling volume of horizon i (m^3), $V_{R,i}$ is root volume (m^3) and $V_{S,i}$ is stone
105 volume (m^3). The nominal sampling volume was calculated from nominal sampling area $A_{N,i}$ (0.0625
106 m^2 for organic horizons, 0.0064 m^2 for mineral horizons) and horizon thickness d_i (m). Root volume
107 was estimated by root fresh mass ($FM_{R,i}$, Mg) assuming a root density of 1 $Mg\ m^{-3}$ (Birouste and others
108 2014). Stone volume was calculated as stone dry mass ($M_{S,i}$, Mg) divided by the density of quartz (2.65
109 $Mg\ m^{-3}$). Similarly, the volume fraction of roots and stones θ_i used in Equation 1 of the main text was
110 calculated as

$$\theta_i = \frac{V_{R,i} + V_{S,i}}{V_{N,i}} \quad (S3)$$

111 The mean volume fraction of roots and stones across all horizons ranged from 0.02 to 0.04 at
112 Klosterhede and from 0.03 to 0.06 at Alptal.

113 Although we used a corer of 30 cm length, the lower boundaries of the lowest horizons (Blr at Alptal
114 and Bs at Klosterhede) measured on the retrieved cores slightly departed from this value. We therefore
115 determined the actual lower boundaries of these horizons on the retrieved cores in the field. We then
116 used these values to determine horizon thickness d_i for calculations of the nominal soil volume

117 (Equation S2), bulk density (Equation S1) and volume fraction of roots and stones (Equation S3). For
118 the calculation of SOC/STN pool sizes (Equation 1 in the main text), however, we mathematically
119 normalized the lower horizon boundaries of to 30 cm before calculating d_i to control for the slight
120 variation in sampling depths. In other words, pool sizes for Blr and Bs horizons were calculated using
121 measured values for bulk density and volume fraction of roots and stones, but lower horizon boundaries
122 were truncated or extended to exactly match 30 cm before Blr/Bs thickness was calculated.

Table S4.1 Horizon thickness, soil dry mass, bulk density (ρ_B), soil organic carbon (SOC) concentrations, soil total nitrogen (STN) concentrations and SOC:STN (mass ratio) in genetic soil horizons at Alptal. Lower-case letters within columns originate from pairwise comparison of horizon means. Means with no letter in common are significantly different (Tukey's HSD; $\alpha = 0.05$). Bold and bold-italic values indicate significant ($P < 0.05$) and marginally significant ($P < 0.1$) post-hoc differences between treatments within a horizon, respectively. Note that post-hoc differences were found for SOC, STN, and SOC:STN despite non-significant interactions. Means ($\pm$ SE) were derived from 6-13 samples per horizon/treatment combination.

	Thickness (cm)	Soil mass (kg m ⁻²)	ρ_B (g cm ⁻³)	SOC (mg g ⁻¹ soil)	STN (mg g ⁻¹ soil)	SOC:STN (mass ratio)
<i>Oi</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>
Control	1.2 $\pm$ 0.2	0.6 $\pm$ 0.1	0.06 $\pm$ 0.01	470.7 $\pm$ 14.6	13.1 $\pm$ 0.4	36.6 $\pm$ 2.0
+ Nitrogen	1.3 $\pm$ 0.3	0.9 $\pm$ 0.2	0.08 $\pm$ 0.02	495.8 $\pm$ 13.1	15.5 $\pm$ 1.0	33.1 $\pm$ 2.0
<i>Oe</i>	<i>ab</i>	<i>ab</i>	<i>a</i>	<i>ab</i>	<i>b</i>	<i>b</i>
Control	1.6 $\pm$ 0.2	0.9 $\pm$ 0.2	0.05 $\pm$ 0.01	514.0 $\pm$ 20.0	19.0 $\pm$ 1.1	27.3 $\pm$ 0.8
+ Nitrogen	2.4 $\pm$ 0.3	2.2 $\pm$ 0.6	0.09 $\pm$ 0.01	458.0 $\pm$ 31.3	20.5 $\pm$ 2.1	23.1 $\pm$ 1.5
<i>Oa</i>	<i>b</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>ab</i>	<i>b</i>
Control	3.5 $\pm$ 0.8	1.8 $\pm$ 0.6	0.06 $\pm$ 0.01	399.6 $\pm$ 20.3	17.2 $\pm$ 1.0	23.6 $\pm$ 0.9
+ Nitrogen	2.7 $\pm$ 0.4	2.7 $\pm$ 0.5	0.11 $\pm$ 0.01	382.1 $\pm$ 25.1	16.0 $\pm$ 1.5	24.5 $\pm$ 2.1
<i>Ah</i>	<i>c</i>	<i>c</i>	<i>b</i>	<i>c</i>	<i>c</i>	<i>c</i>
Control	8.0 $\pm$ 0.7	22.8 $\pm$ 3.7	0.30 $\pm$ 0.03	221.2 $\pm$ 31.6	10.3 $\pm$ 1.4	21.3 $\pm$ 0.8
+ Nitrogen	6.5 $\pm$ 0.9	15.5 $\pm$ 3.5	0.25 $\pm$ 0.03	169.0 $\pm$ 17.2	8.3 $\pm$ 0.8	20.3 $\pm$ 0.5
<i>Bl</i>	<i>c</i>	<i>d</i>	<i>c</i>	<i>d</i>	<i>d</i>	<i>c</i>
Control	9.5 $\pm$ 1.1	68.5 $\pm$ 11.2	0.74 $\pm$ 0.07	44.2 $\pm$ 6.0	2.4 $\pm$ 0.3	18.5 $\pm$ 0.6
+ Nitrogen	9.1 $\pm$ 0.8	54.4 $\pm$ 6.3	0.63 $\pm$ 0.07	55.8 $\pm$ 6.5	2.9 $\pm$ 0.3	19.2 $\pm$ 0.4
<i>Blr</i> ^a	<i>d</i>	<i>d</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>c</i>
Control	11.5 $\pm$ 1.5	95.9 $\pm$ 8.2	0.93 $\pm$ 0.05	36.3 $\pm$ 4.2	1.8 $\pm$ 0.2	20.4 $\pm$ 0.8
+ Nitrogen	13.9 $\pm$ 0.9	80.8 $\pm$ 10.0	0.82 $\pm$ 0.07	32.1 $\pm$ 2.5	1.7 $\pm$ 0.1	19.5 $\pm$ 1.0
Sign. effects	Horizon	Horizon, +N x Horizon	Horizon, +N x Horizon	Horizon	Horizon	Horizon

^a Lower horizon boundary of *Blr* horizons was normalized to 30 cm for calculation of horizon thickness. Soil mass and bulk density were calculated using original sampling depths of 30.0 $\pm$ 2.1 cm and 26.3 $\pm$ 1.0 cm from top of mineral soil in control and +N plots, respectively.

Table S4.2 Horizon thickness, soil dry mass, bulk density (ρ_B), soil organic carbon (SOC) concentrations, soil total nitrogen (STN) concentrations and SOC:STN (mass ratio) in genetic soil horizons at Klosterhede. Lower-case letters within columns originate from pairwise comparison of horizon means. Means with no letter in common are significantly different (Tukey's HSD; $\alpha = 0.05$). Bold and bold-italic values indicate significant ($P < 0.05$) and marginally significant ($P < 0.1$) post-hoc differences between treatments within a horizon, respectively. Note that post-hoc differences were found for bulk density, STN in mineral horizons, and SOC:STN despite non-significant interactions. Means ($\pm$ SE) were derived from 10-12 samples per horizon/treatment combination.

	Thickness (cm)	Soil mass (kg m ⁻²)	ρ_B (g cm ⁻³)	SOC (mg g ⁻¹ soil)	STN (mg g ⁻¹ soil)	SOC:STN (mass ratio)
Oe	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i> [†]	<i>a</i> [†]	<i>a</i>
Control	5.1 $\pm$ 0.4	4.9 $\pm$ 0.5	0.10 $\pm$ 0.01	500.3 $\pm$ 4.8	15.6 $\pm$ 0.6	32.6 $\pm$ 1.2
+ Nitrogen	5.5 $\pm$ 0.3	6.6 $\pm$ 0.6	0.12 $\pm$ 0.01	485.2 $\pm$ 4.3	15.5 $\pm$ 0.3	31.4 $\pm$ 0.7
Oa	<i>b</i>	<i>a</i>	<i>a</i>	<i>b</i> [†]	<i>b</i> [†]	<i>b</i>
Control	3.2 $\pm$ 0.6	3.8 $\pm$ 0.3	0.17 $\pm$ 0.03	394.9 $\pm$ 9.2	10.2 $\pm$ 0.4	39.2 $\pm$ 1.6
+ Nitrogen	3.4 $\pm$ 0.4	4.0 $\pm$ 0.5	0.13 $\pm$ 0.02	413.3 $\pm$ 8.3	11.2 $\pm$ 0.4	37.4 $\pm$ 1.2
AE	<i>c</i>	<i>b</i>	<i>b</i>	<i>a</i> [‡]	<i>a</i> [‡]	<i>c</i>
Control	8.5 $\pm$ 0.5	76.5 $\pm$ 5.8	0.92 $\pm$ 0.03	47.7 $\pm$ 3.5	0.9 $\pm$ 0.1	56.9 $\pm$ 2.8
+ Nitrogen	6.5 $\pm$ 0.4	50.5 $\pm$ 4.1	0.81 $\pm$ 0.04	53.9 $\pm$ 5.5	1.1 $\pm$ 0.1	52.2 $\pm$ 2.7
E	<i>cd</i>	<i>b</i>	<i>c</i>	<i>b</i> [‡]	<i>b</i> [‡]	<i>c</i>
Control	7.4 $\pm$ 0.6	77.2 $\pm$ 7.2	1.07 $\pm$ 0.04	27.4 $\pm$ 2.1	0.5 $\pm$ 0.0	50.6 $\pm$ 1.9
+ Nitrogen	6.9 $\pm$ 0.4	64.2 $\pm$ 4.2	0.98 $\pm$ 0.03	26.6 $\pm$ 1.6	0.5 $\pm$ 0.0	49.6 $\pm$ 1.8
Bh	<i>ad</i>	<i>c</i>	<i>b</i>	<i>a</i> [‡]	<i>c</i> [‡]	<i>b</i>
Control	5.1 $\pm$ 0.2	39.5 $\pm$ 2.2	0.81 $\pm$ 0.04	58.3 $\pm$ 2.6	1.5 $\pm$ 0.1	39.3 $\pm$ 0.9
+ Nitrogen	6.1 $\pm$ 0.5	47.4 $\pm$ 4.5	0.80 $\pm$ 0.04	56.6 $\pm$ 2.5	1.4 $\pm$ 0.1	41.1 $\pm$ 1.3
Bs^a	<i>e</i>	<i>d</i>	<i>c</i>	<i>b</i> [‡]	<i>d</i> [‡]	<i>b</i>
Control	9.6 $\pm$ 1.2	104.8 $\pm$ 9.8	0.97 $\pm$ 0.04	29.4 $\pm$ 1.7	0.7 $\pm$ 0.1	42.0 $\pm$ 1.3
+ Nitrogen	10.6 $\pm$ 0.9	94.7 $\pm$ 4.5	0.96 $\pm$ 0.03	30.5 $\pm$ 1.2	0.8 $\pm$ 0.0	41.3 $\pm$ 1.6
Sign. effects	Horizon, +N x Horizon ^b	+N ^c , Horizon, +N x Horizon	+N ^d , Horizon	Org: Horizon, +N x Horizon Min: Horizon	Org: Horizon, +N x Horizon Min: Horizon	Horizon

^a Lower horizon boundary of Bs horizons was normalized to 30 cm for calculation of horizon thickness. Soil mass and bulk density were calculated for original sampling depths of 31.6 $\pm$ 1.3 cm and 29.7 $\pm$ 0.8 cm from top of mineral soil in control and +N treatment plots, respectively. Marginally significant main effects and treatment x horizon interactions (^bP=0.089, ^cP=0.085, ^dP=0.077).

[†] Lower-case letters indicate significant post-hoc differences between organic horizons.

[‡] Lower-case letters indicate significant post-hoc differences between mineral horizons.

141 **Table S4.3** Summary of N-induced changes of direct controls of SOC and STN pools in Oe and A
 142 horizons. Black arrows indicate a significant N-induced increase/decrease compared to controls
 143 ($P < 0.05$). Grey arrows indicate non-significant trends.

144

	Alptal		Klosterhede	
	Oe horizon	Ah horizon	Oe horizon	AE horizon
Thickness	↑	↓	↑	↓
Bulk density	↑	↓	↑	↓
SOC concentration	↓	↓	↓	↑
STN concentration	↑	↓	↓	↑
SOC pool	↑	↓	↑	↓
STN pool	↑	↓	↑	↓

145 **Appendix 5:** Total belowground C and N pools – Supplementary Table

146 **Table S5.1.** Total belowground C and N pools at Alptal and Klosterhede. P-values are derived from
 147 linear mixed models including block and replicate within treatment and block as random effects (see
 148 Methods sections for details). In addition, bold and bold-italic values indicate significant ($P < 0.05$) and
 149 marginally significant ($P < 0.1$) differences between treatments, respectively. Means ($\pm$ SE) were
 150 derived from 12 samples except for the control plots at Klosterhede (10 samples).

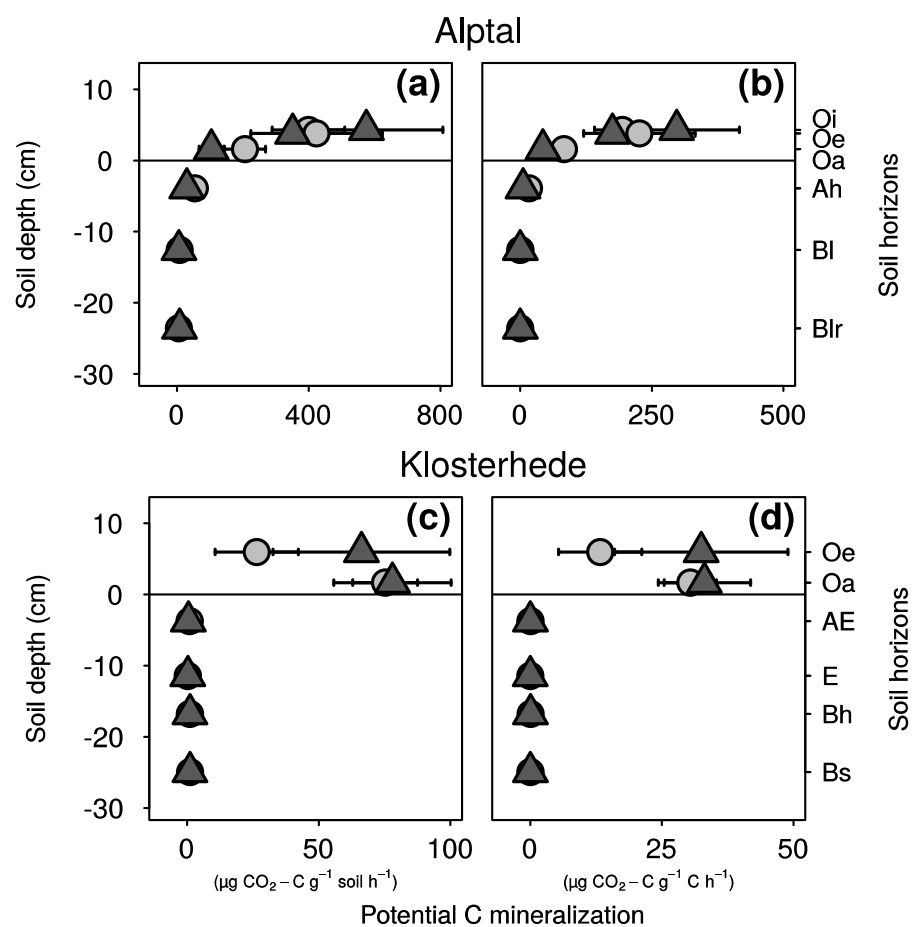
151

	Alptal		Klosterhede	
	C pool (kg m ⁻²)	N pool (kg m ⁻²)	C pool (kg m ⁻²)	N pool (kg m ⁻²)
<i>Soil</i>				
Control	11.91 $\pm$ 0.78	0.595 $\pm$ 0.033	14.23 $\pm$ 0.55	0.332 $\pm$ 0.014
+ Nitrogen	10.41 $\pm$ 0.79	0.526 $\pm$ 0.035	14.87 $\pm$ 0.36	0.374 $\pm$ 0.014
P	0.181	0.172	0.381	0.055
<i>Fine roots (<2mm)</i>				
Control	0.22 $\pm$ 0.04	0.004 $\pm$ 0.001	0.21 $\pm$ 0.02	0.003 $\pm$ 0.000
+ Nitrogen	0.24 $\pm$ 0.04	0.004 $\pm$ 0.001	0.28 $\pm$ 0.03	0.006 $\pm$ 0.001
P	0.974	0.884	0.038	<0.001
<i>Soil and fine roots</i>				
Control	12.13 $\pm$ 0.80	0.598 $\pm$ 0.034	14.43 $\pm$ 0.56	0.335 $\pm$ 0.014
+ Nitrogen	10.65 $\pm$ 0.80	0.530 $\pm$ 0.035	15.15 $\pm$ 0.36	0.380 $\pm$ 0.014
P	0.202	0.180	0.332	0.044

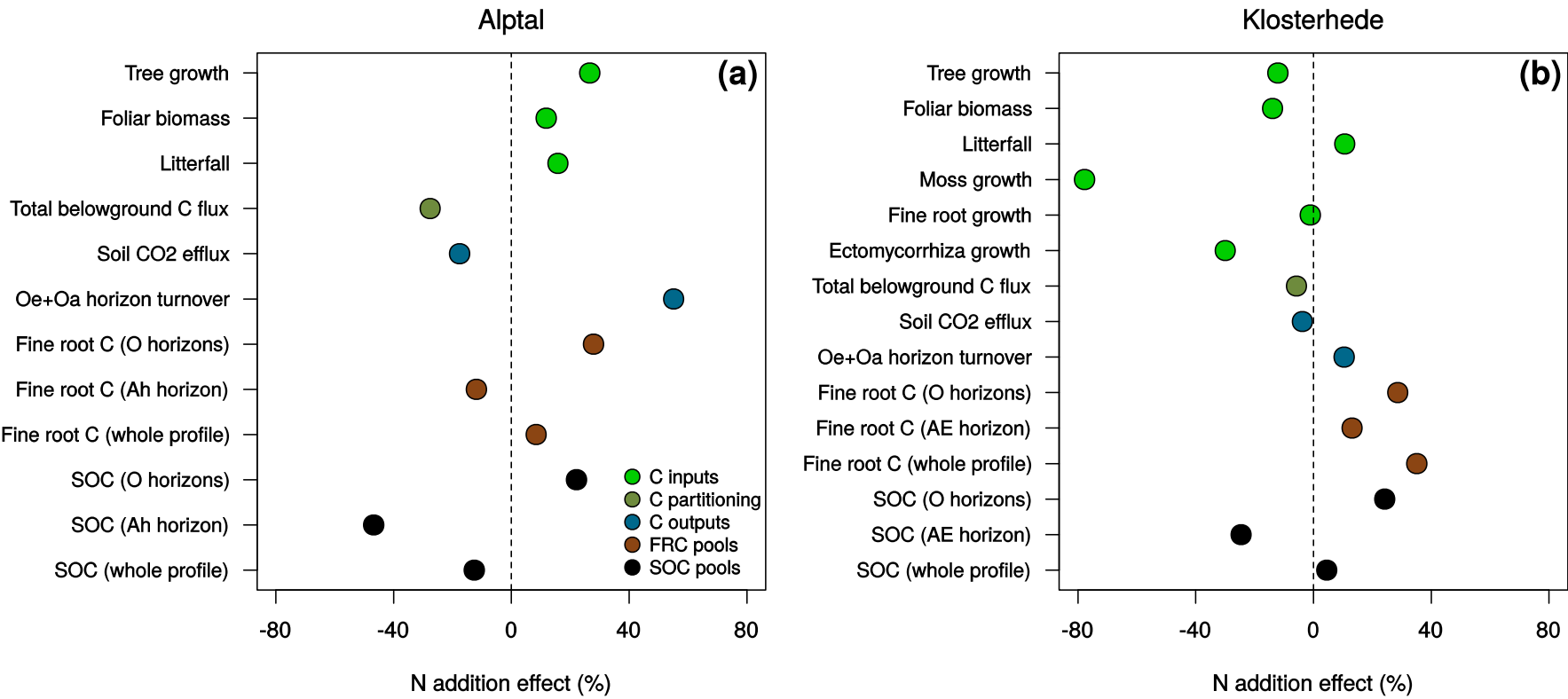
152 **Appendix S6:** Potential C mineralization rates and ecosystem-level C cycling

153 For the measurement of potential C mineralization rates, 5-20g of field-moist soil was evenly spread out over the bottom of cylindrical
154 PVC-tubes (10 cm diameter), adjusted to 60% maximum water holding capacity and incubated at 10°C for three days approximating the
155 mean annual temperature at our sites. During incubation, PVC-tubes were covered with Parafilm® to allow for gas exchange but prevent
156 water loss. After incubation, respiration rates were measured at room temperature with a portable infrared gas analyzer (LI-8100A, LI-COR
157 Bioscience, Lincoln, NE, U.S.) equipped with a 10-cm diameter survey chamber (8100-102, LI-COR Bioscience). For each measurement,
158 the survey chamber was placed on top of the PVC-tube to create a closed-chamber system with a defined headspace volume (1134.8 cm³).
159 Then, CO₂ concentrations in the closed-chamber system were monitored over two minutes (pre-purge: 30s, post-purge: 10s) with a reading
160 taken every second. Mixing and pressure equalization of chamber volume during measurements was ensured by the LI-COR system. Fluxes
161 of CO₂ (μmol CO₂ m⁻² s⁻¹) were obtained by fitting non-linear regression models to the CO₂ data using the manufacturers FV8100v3.1.1
162 software package. The resulting area-based fluxes were normalized to soil dry mass and SOC concentrations (μg CO₂-C g⁻¹ soil h⁻¹ and μg
163 CO₂-C g⁻¹ soil C h⁻¹) using the molar mass of C (12 g mol⁻¹), the area over which the soil was spread out (0.0078 m²) and measurements of
164 gravimetric water content and SOC concentrations.

165 **Figure S6.1** Potential C mineralization rates per g soil (a,c) and per g SOC (b,d) in genetic soil horizons at Alptal (upper panels) and
 166 Klosterhede (lower panels). Light grey circles depict data from controls plots, dark gray triangles depict data from N addition plots.
 167 Lower-case letters next to the right border of each plate originate from pairwise comparison of horizon means. Means with no letter in
 168 common are significantly different (Tukey's HSD; $\alpha = 0.05$). Note that there was a significant post-hoc difference between treatments for
 169 specific respiration rates in the Oe horizon at Klosterhede (d, $P < 0.001$). Means ($\pm$ SE) were derived from 4 samples per horizon/treatment
 170 combination.



172 **Figure S6.2** Effects of N addition on selected C cycling parameters at Alptal (a) and Klosterhede (b). See Tables S6.1 and S6.2 for details.



174 **Table S6.1** Carbon inputs, C partitioning, C outputs and belowground C pools in response to long-term N addition at Alptal.

175

	Units	Control	+ Nitrogen	% Change	Level of significance	Time	Reference
C inputs							
Tree growth rates (Basal area increment) ^A	mm ² y ⁻¹	632.0	800.5	27	n.a.	1960-2008	Krause and others 2012
Foliar biomass	mg needle ⁻¹	5.5	6.1	12	n.a.	1995-2009	Krause and others 2012
Foliar litterfall ^B	g C m ⁻² y ⁻¹	113.3	131.2	16	n.a.	1995-2008 ^C	P. Schleppi unpublished results
C partitioning							
Total belowground C flux (TBCF) ^D	g C m ⁻² y ⁻¹	300.0	217.1	-28	n.a.	1995-2012	This study
Belowground net primary production (BNPP) ^E	g C m ⁻² y ⁻¹	126.0	91.2	-28	n.a.	1995-2012	This study
C outputs							
In-situ soil CO ₂ efflux	g C m ⁻² y ⁻¹	421.0	347.0	-18	n.a.	2007-2012	Krause and others 2013
Methane flux	g C m ⁻² y ⁻¹	0.0	0.1	1100	n.a.	2007-2012	Krause and others 2013
Potential soil respiration – Oi	µg CO ₂ -C g ⁻¹ soil h ⁻¹	399.3	575.2	44	n.s. (P= 0.718, Dunnett's test)	2014	This study
Potential soil respiration – Oe	µg CO ₂ -C g ⁻¹ soil h ⁻¹	423.9	351.3	-17	n.s. (P= 0.589, Dunnett's test)	2014	This study
Potential soil respiration – Oa	µg CO ₂ -C g ⁻¹ soil h ⁻¹	206.6	104.3	-50	n.s. (P= 0.221, Dunnett's test)	2014	This study
Potential soil respiration – Ah	µg CO ₂ -C g ⁻¹ soil h ⁻¹	54.0	29.7	-45	n.s. (P= 0.430, Dunnett's test)	2014	This study
Potential soil respiration – Bl	µg CO ₂ -C g ⁻¹ soil h ⁻¹	8.3	6.0	-28	n.s. (P= 0.603, Dunnett's test)	2014	This study
Potential soil respiration – Blr	µg CO ₂ -C g ⁻¹ soil h ⁻¹	5.9	7.9	34	n.s. (P= 0.387, Dunnett's test)	2014	This study
Oe+Oa horizon turnover ^F	years	11.9	18.5	55	n.a.	1992-2014	This study
C pools							
Fine root C pool in Ohorizons	g C m ⁻²	61.1	78.2	28	n.s. (P= 0.978, Dunnett's test)	2014	This study
Fine root C pool in Ah horizon	g C m ⁻²	138.1	121.7	-12	n.s. (P= 0.588, Dunnett's test)	2014	This study
Fine root C pool in Bl horizon	g C m ⁻²	16.8	24.9	48	n.s. (P= 0.997, Dunnett's test)	2014	This study
Fine root C pool in Blr horizon	g C m ⁻²	2.9	12.8	335	n.s. (P= 0.200, Dunnett's test)	2014	This study
Fine root C pool in whole profile	g C m ⁻²	219.0	237.5	8	n.s. (P= 0.974, mixed model)	2014	This study
Soil C pool in O horizons	kg C m ⁻²	1.3	1.6	22	n.s. (P= 0.304, Dunnett's test)	2014	This study
Soil C pool in Ah horizon	kg C m ⁻²	4.4	2.4	-47	** (P= 0.002, Dunnett's test)	2014	This study
Soil C pool in Bl horizon	kg C m ⁻²	2.6	3.0	15	n.s. (P= 0.706, Dunnett's test)	2014	This study
Soil C pool in Blr horizon	kg C m ⁻²	3.2	3.3	2	n.s. (P= 0.752, Dunnett's test)	2014	This study
Soil C pool in whole profile	kg C m ⁻²	11.9	10.4	-13	n.s. (P= 0.181, mixed model)	2014	This study

^A "Control" refers to the average annual BAI increments on the future N addition plots from 1960-1994 before N additions started, "+ Nitrogen" refers to BAI increments of N addition plots within the period of N additions, i.e. from 1995 to 2008.

^B Assuming 50% of litter dry mass is C.

^C Excluding data from 2003 where litterfall was unusually high in N addition plots due to a bark beetle infestation.

^D Calculated as in-situ soil CO₂ efflux minus foliar litterfall (see Raich & Nadelhoffer 1989, Litton & Giardina 2008).

^E Assuming that 0.42 of TBCF is allocated to BNPP at MAT=6°C (Litton & Giardina 2008).

^F Calculated as Oe+Oa mass divided by foliar litterfall (see Zak et al. 2008).

n.a. = not analyzed

n.s. = not significant at P<0.05

176 Annual tree growth rates were calculated from basal area increments determined on tree rings (Krause and others 2012). Estimates of foliar
177 biomass were derived from needle samples taken yearly from the seventh whorl from five tree tops per plot. For each of the five most recent
178 needle age classes, 120 needles were dried to constant weight (65°C) before needle dry mass was determined (Schleppi and others 1999;
179 Krause and others 2012). Foliar litterfall was sampled every second week from four collectors (0.12 m²) per plot (Schleppi and others 1999;
180 Krause and others 2013). Soil CO₂ efflux and CH₄-C fluxes were measured by repeated sampling of the headspace of closed PVC chambers
181 (height: 30 cm, inner diameter: 32 cm) in each plot (Krause and others 2013).

182 **Table S6.2** Carbon inputs, C partitioning, C outputs and belowground C pools in response to long-term N addition at Klosterhede.

C inputs							
Tree growth rates (Basal area increment)	m ² ha ⁻¹ y ⁻¹	0.865	0.761	-12	n.a.	1992-2014	Ginzburg (2014)
Tree growth rates (Basal area increment)	m ² ha ⁻¹ y ⁻¹	0.895	0.680	-24	n.a.	2004-2014	Ginzburg (2014)
Foliar biomass	mg needle ⁻¹	4.527	3.897	-14	n.a.	1992-2009	P. Gundersen unpublished results
Foliar litterfall ^A	g C m ⁻² y ⁻¹	112.1	123.9	11	n.a.	1992-2010	Ginzburg (2014)
Moss growth	g C m ⁻² y ⁻¹	9.0	2.0	-78	n.a.	2013	Ginzburg (2014)
Fine root growth	g m ⁻² y ⁻¹	177	175	-1	n.s. (P=0.7, paired t-test)	2007-2008	Ginzburg (2014)
EM growth	g m ⁻² y ⁻¹	10	7	-30	n.s. (P=0.3, t-test)	2010	Ginzburg (2014)
C partitioning							
Total belowground C flux (TBCF) ^B	g C m ⁻² y ⁻¹	775.6	730.3	-6	n.a.	1992-2010	This study
Belowground net primary production (BNPP) ^C	g C m ⁻² y ⁻¹	349.0	328.6	-6	n.a.	1992-2010	This study
C outputs							
In-situ soil CO ₂ efflux	mg CO ₂ -C m ⁻² h ⁻¹	101.3	97.5	-4	n.a.	2002-2003	Ginzburg (2014)
In-situ soil CO ₂ efflux – interpolation ^D	mg CO ₂ -C m ⁻² h ⁻¹	103.3	101.7	-2	n.a.	2002-2003	Ginzburg (2014)
Litter decomp. – exogenous N, 0-18 mon.	% accum. mass loss	23.2	20.7	-11	n.a.	2001-2006	Ginzburg (2014)
Litter decomp. – exogenous N, 24-60 m.	% accum. mass loss	60.8	58.2	-4	n.a.	2001-2006	Ginzburg (2014)
Litter decomp. – exogenous N, 0-18 m.	% mass loss month ⁻¹	2.1	1.8	-13	n.a.	2001-2006	Ginzburg (2014)
Litter decomp. – exogenous N, 24-60 m.	% mass loss month ⁻¹	1.1	1.2	9	n.a.	2001-2006	Ginzburg (2014)
Litter decomp. – endogenous N, 0-18 m.	% accum. mass loss	20.3	23.5	16	n.a.	2001-2006	Ginzburg (2014)
Litter decomp. – endogenous N, 24-60 m.	% accum. mass loss	60.3	58.7	-3	n.a.	2001-2006	Ginzburg (2014)
Litter decomp. – endogenous N, 0-18 m.	% mass loss month ⁻¹	1.9	2.1	12	n.a.	2001-2006	Ginzburg (2014)
Litter decomp. – endogenous N, 24-60 m.	% mass loss month ⁻¹	1.3	1.0	-23	n.a.	2001-2006	Ginzburg (2014)
Potential C mineralization – LF (Oie)	mg CO ₂ -C g ⁻¹ soil C h ⁻¹	0.031	0.040	29	n.s.	2003	Ginzburg (2014)
Potential C mineralization – H (Oa)	mg CO ₂ -C g ⁻¹ soil C h ⁻¹	0.020	0.017	-14	n.s.	2003	Ginzburg (2014)
Potential C mineralization – Min	mg CO ₂ -C g ⁻¹ soil C h ⁻¹	0.011	0.010	-6	n.s.	2003	Ginzburg (2014)
Potential C mineralization – LF (Oie)	mg CO ₂ -C g ⁻¹ soil C h ⁻¹	0.022	0.019	-15	n.s.	2013	Ginzburg (2014)
Potential C mineralization – H (Oa)	mg CO ₂ -C g ⁻¹ soil C h ⁻¹	0.010	0.009	-13	n.s.	2013	Ginzburg (2014)
Potential C mineralization – Min	mg CO ₂ -C g ⁻¹ soil C h ⁻¹	0.016	0.019	15	n.s.	2013	Ginzburg (2014)

^A Assuming 50% of litter dry mass is C.

^B Calculated as in-situ soil respiration (converted to g C m⁻² y⁻¹) minus foliar litterfall (see Raich & Nadelhoffer 1989, Litton & Giardina 2008).

^C Assuming that 0.45 of TBCF is allocated to BNPP at MAT=10°C (Litton & Giardina 2008).

^D In-situ soil respiration was measured on different occasions during 2002 (April, June, Oktober, November, December) and 2003 (February, June, August). In addition to reporting average values from these measurements, we linearly interpolated fluxes between measurements and calculated average fluxes from this interpolated dataset.

	Units	Control	+ Nitrogen	% Change	Level of significance	Time	Reference
C outputs – continued							
Potential C mineralization – Oe	$\mu\text{g CO}_2\text{-C g}^{-1} \text{ soil h}^{-1}$	26.5	66.2	150	n.s. (P=0.140, Dunnett's test)	2014	This study
Potential C mineralization – Oa	$\mu\text{g CO}_2\text{-C g}^{-1} \text{ soil h}^{-1}$	75.2	78.0	4	n.s. (P=0.907, Dunnett's test)	2014	This study
Potential C mineralization – AE	$\mu\text{g CO}_2\text{-C g}^{-1} \text{ soil h}^{-1}$	0.97	0.51	-47	n.s. (P=0.170, Dunnett's test)	2014	This study
Potential C mineralization – E	$\mu\text{g CO}_2\text{-C g}^{-1} \text{ soil h}^{-1}$	0.25	0.28	12	n.s. (P=0.912, Dunnett's test)	2014	This study
Potential C mineralization – Bh	$\mu\text{g CO}_2\text{-C g}^{-1} \text{ soil h}^{-1}$	1.06	1.10	4	n.s. (P=0.937, Dunnett's test)	2014	This study
Potential C mineralization – Bs	$\mu\text{g CO}_2\text{-C g}^{-1} \text{ soil h}^{-1}$	1.14	1.10	-4	n.s. (P=0.720, Dunnett's test)	2014	This study
Oe+Oa horizon turnover ^E	years	38.5	42.5	10	n.a.	1992-2014	This study
C pools							
Moss C pool	g C m^{-2}	72.0	15.0	-79	n.a.	2013	Ginzburg (2014)
Fine root C pool in O horizons	g C m^{-2}	89.0	114.5	29	n.s. (P=0.123, Dunnett's test)	2014	This study
Fine root C pool in AE horizon	g C m^{-2}	43.6	49.3	13	n.s. (P=0.624, Dunnett's test)	2014	This study
Fine root C pool in E horizon	g C m^{-2}	24.0	30.3	26	n.s. (P=0.294, Dunnett's test)	2014	This study
Fine root C pool in Bh horizon	g C m^{-2}	23.4	26.6	14	n.s. (P=0.475, Dunnett's test)	2014	This study
Fine root C pool in Bs horizon	g C m^{-2}	39.3	59.1	50	n.s. (P=0.258, Dunnett's test)	2014	This study
Fine root C pool in whole profile	g C m^{-2}	207.1	279.8	35	* (P=0.038, mixed model)	2014	This study
Soil C pool in O horizons	kg C m^{-2}	3.9	4.9	24	* (P=0.037, Dunnett's test)	2014	This study
Soil C pool in AE horizon	kg C m^{-2}	3.5	2.6	-25	* (P=0.024, Dunnett's test)	2014	This study
Soil C pool in E horizon	kg C m^{-2}	2.2	1.7	-24	n.s. (P=0.191 Dunnett's test)	2014	This study
Soil C pool in Bh horizon	kg C m^{-2}	2.3	2.7	19	n.s. (P=0.357, Dunnett's test)	2014	This study
Soil C pool in Bs horizon	kg C m^{-2}	2.6	2.9	13	n.s. (P=0.286, Dunnett's test)	2014	This study
Soil C pool in whole profile	kg C m^{-2}	14.2	14.9	5	n.s. (P=0.381, mixed model)	2014	This study

^E Calculated as Oe+Oa mass divided by foliar litterfall (see Zak et al. 2008).

n.a. = not analyzed

n.s. = not significant at P<0.05

186 Tree growth rates were calculated from changes in basal area increment (BAI), which were derived from annual measurements of tree
 187 diameter at breast height (DBH) since 1990 (Ginzburg 2014). Estimated of foliar biomass was derived from 100 current year needles taken
 188 from the seventh whorl of 5 randomly selected trees per plot and year (P. Gundersen, pers.communication). Litterfall was collected monthly

189 in 10 traps per plot in the years 1991-1995, 2002 and 2010. The foliar fraction was sorted out, dried at 55°C to constant mass and weighted
190 (Ginzburg 2014).

191 Moss growth was estimated by a three step-procedure: First, moss cover (%) of control and N addition plots was visually estimated in
192 1991-1995 and in 2013. Then, moss cover was converted into moss biomass using the cover-biomass relationships measured in three fully
193 covered 10 cm x 10 cm squares taken from the control plots. Finally, moss biomass was divided by an assumed lifetime of 8 years to
194 calculated annual moss growth (Ginzburg 2014).

195 Fine root growth was estimated during 2007-2008 using ingrowth cores (Ginzburg 2014)7). Growth of ectomycorrhizal fungi was estimated
196 in 2009-2010 using ingrowth bags (mesh sizes 50 µm; (Ginzburg 2014). In-situ soil C efflux rates were measured during 2002 in 15 PVC
197 collars per treatment with continuous-flow chamber attached to an infrared gas analyzer (Ginzburg 2014).

198 Litter decomposition was measured after 10 years of N addition treatments in a reciprocal transplant experiment to separate the effects of N
199 on litter traits (endogenous N effect) and soil environment (exogenous N effect). Therefore, dried needle litter from control and N addition
200 plots was placed in nylon mesh litterbags (0.5 mm x 1mm mesh size) and a total of 360 litterbags were deployed in control and N addition
201 plots in 2001. Sets of 5 replicated litterbags were collected after 6, 12, 18, 24, 32 and 60 months from 3 random locations within control and
202 N addition plots. Replicates were pooled to 3 composite samples per treatment before dry mass was determined (Ginzburg 2014). Potential C
203 mineralization rates were measured in 2003 and 2013 on six and four replicates per treatment and soil layer (LF, H, Min) using a
204 Respirometer (Ginzburg 2014).

205 **Appendix S7:** Statistical considerations regarding the experimental layout at Klosterhede

206 The original experimental layout at Klosterhede posed some challenges before the experiment could be
207 analyzed as a split block design. Originally, the experiment included one single area over which N has
208 been applied, framed by two control areas (see Figure S7.1). As such, there are strictly speaking just
209 two plots at Klosterhede, one which received N and one that did not, even though the control plot
210 consists of two areas which are located on two of the sides of the N addition plot. If we had analyzed
211 the experiment as suggested by the original spatial arrangement of plots, we would have been able
212 to use inferential statistics to test for differences between treatments at that special location, but
213 inference beyond the experimental site would not have been valid. However, the main aim of our study
214 was to learn (infer) from this unique long-term experiment how decadal N addition affects C pools in
215 temperate forests generally, and we feel that our reinterpretation of the original study design allows us
216 to do so.

217 Thus, we first divided the original N addition and control plots into four subplots each and then blocked
218 one N subplot with an adjacent control subplot (see Figure S7.1). This was not done to uncritically
219 increase replication, but to *post-hoc* mimick a blocked design, similar to that employed at Alptal. In this
220 view, each block at Klosterhede contains two experimental units (henceforth termed “plots”) to which
221 treatments have been applied. The resulting analytical approach is similar to a situation in which the
222 treatments would have been applied at a block level in the first place. Hence, we employed linear
223 (mixed) models to assess effects of horizon and N addition treatment at Klosterhede.

224 A main caveat remains: Treatments have not been assigned randomly to plots within blocks. However,
225 we stress that it is not the lack of random assignment *per se* that is of concern as “randomization is
226 simply a way of achieving interspersion in a way that eliminates the possibility of bias [...]” (Hurlbert
227 1984, 192). Even strict randomization would have produced the employed layout with a probability of
228 6.25%, assuming a 50% chance for each plot to be assigned to one of the two treatments within a block.

229 Rather, it is the lack of interspersions/the spatial segregation of treatments that can lead to a lack of
230 independence of errors, thus would have restricted our ability to exactly know if the probability of type
231 I errors were 5% as assumed in the analysis, and precluded inference (Hurlbert 1984, 192). This is
232 because interspersions of treatments controls for (1) initial differences or gradients between plots within
233 a block and (2) for differences between plots that either arise or intensify during the experiment as a
234 result of non-demonic intrusion (Hurlbert 1984, 195).

235 For lack of interspersions not to invalidate our analysis, we have to show that (1) no within-block
236 gradients existed before treatments were applied and (2) that any non-demonic intrusion did not
237 selectively affect the spatially segregated N addition treatments so that no within-block gradients arose
238 during the experiment. We believe that strong gradients did not exist before the start of the experiment
239 for the following reasons:

240 (1) The parent material at Klosterhede has been judged to be a “homogenous sandy, nutrient
241 poor glacio-fluvial outwash plain” (Gundersen and Rasmussen 1995). This indicates that strong
242 gradients in soil properties due to variations in parent material are unlikely.

243 (2) The site has been ploughed prior to forest establishment. Thus it is reasonable to assume a
244 high degree of homogeneity in initial soil properties compared to sites where natural soil
245 development progressed unaltered.

246 We further employed two unconstrained ordination techniques (DCA, PCA) to evaluate if strong
247 gradients were present across the experimental area (and thus within blocks) at the time of sampling,
248 either as a consequence of pre-existing gradients or due to “non-demonic intrusion” during the
249 experiment. Specifically, we tested if

250 (Objective 1) multivariate gradients were detectable across the experimental area in variables
251 not significantly affected by N addition treatment (mineral soil texture, EOC, mineral soil

CEC). For this analysis, we used data from mineral horizons of all eight plots, where texture and CEC were measured.

(Objective 2) multivariate gradients were detectable in variables significantly affected by N addition treatment in at least one horizon (thickness, bulk density, %C, NH₄-N, NO₃-N, EON). Here, we only used data from the four control plots. The spatial arrangement of the control plots (see Figure S6.1) allowed us to check for gradients independent of N addition across the experimental area at the time of sampling.

First, we used detrended correspondence analysis (DCA) to investigate the length of possible gradients within each horizon. The length of DCA axes indicates the length of ecological gradients (Hill and Gauch 1980) which can be interpreted as the “beta diversity in community composition (the extent of species turnover) along the individual independent gradients [...]” (Lepš and Šmilauer 2003, 51) in case the response is multivariate species abundance. However, the reasoning also applies to any response for which unimodal distribution is assumed. If the DCA axis is smaller than 3 SD units, the ecological gradient is regarded as “short”. If it is larger than 4 SD units, the “turnover” in the multivariate response is high, the gradient is regarded as “long”(Lepš and Šmilauer 2003).

Second, we conducted two PCAs for each soil horizon. The first PCA was based on soil texture, EOC and CEC and was carried out for mineral horizons of all eight plots (Objective 1). The second PCA was based on thickness, bulk density, %C, NH₄-N, NO₃-N and EON and was carried out for all horizons of the four control plots (Objective 2).

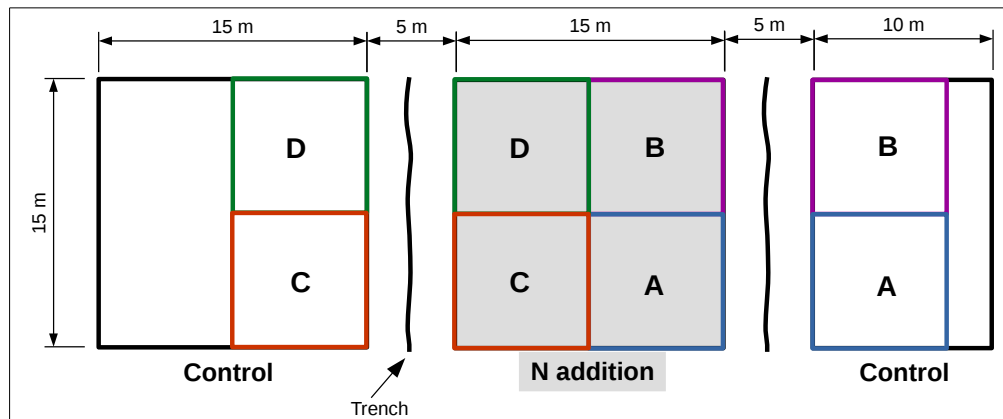
With regard to Objective 1, we found only short within-horizon gradients across all eight plots as indicated by the lengths of the first DCA axes (<0.6 SD). Horizon-specific PCAs further revealed that the first three axes accounted for ~90% of the variance within each horizon, and that plots generally did not separate along PCA axes. The only exception was found within the AE horizon where plots of

275 block D were significantly separated along PC3 (11.5% of total variance). This was driven by a higher
276 CEC in this block.

277 With regard to Objective 2, DCA also revealed short gradients (<0.7 SD). Within this relatively low
278 spatial variation, some control plots were separated along PCA axes when the variables affected by N
279 addition were considered. These separations, however, were horizon- and block-specific: In the Oe
280 horizon, the control plot of block C separated along PC1 (48.3% of total variance) which was driven by
281 on average higher amounts of $\text{NH}_4\text{-N}$ and EON as well as a smaller horizon thickness. The AE horizon
282 of the control plot in block A was relatively thick on average, which drove separation along PC2
283 (58.1% of total variance). Further, the Bh horizon of block B had a lower bulk density on average and
284 therefore was separated along PC2 in the respective PCA (52.9% of total variance).

285 In summary, we did not find strong gradients across plots in variables unaffected by N addition
286 (Objective 1). Similarly, we did not find strong, consistent within-horizon gradients between control
287 plots (Objective 2). Still, slight differences between blocks indicate that some caution is required in
288 interpreting the effect of N addition on horizon thickness and derived quantities. We conclude that
289 slight differences in horizon thickness unrelated to N addition might have been present at Klosterhede,
290 but do not affect our general conclusions, i.e. that N addition resulted in a vertical redistribution of
291 SOC pools from AE to Oe horizons.

292 **Figure S7.1** Sketch of the experimental set-up at Klosterhede modified from Gundersen and
293 Rasmussen (1995). The layout consists of one area over which N has been applied, framed by two
294 control areas. Nitrogen additions started in February 1992. Quadrants sharing the same uppercase letter
295 were treated as block in the statistical analyses.



- Birouste M, Zamora-Ledezma E, Bossard C, Pérez-Ramos IM, Roumet C. 2014. Measurement of fine root tissue density: a comparison of three methods reveals the potential of root dry matter content. *Plant Soil* 374:299–313.
- Ginzburg SO. 2014. Nitrogen Deposition Effects on Soil Carbon Dynamics in Temperate Forests. Unpublished Doctoral Thesis. University of Copenhagen, Denmark.
- Gundersen P, Rasmussen L. 1995. Nitrogen mobility in a nitrogen limited forest at Klosterhede, Denmark, examined by NH_4NO_3 addition. *For Ecol Manag* 71:75–88.
- Hurlbert SH. 1984. Pseudoreplication and the Design of Ecological Field Experiments. *Ecol Monogr* 54:187–211.
- Krause K, Cherubini P, Bugmann H, Schleppi P. 2012. Growth enhancement of *Picea abies* trees under long-term, low-dose N addition is due to morphological more than to physiological changes. *Tree Physiol* 32:1471–81.
- Krause K, Niklaus PA, Schleppi P. 2013. Soil-atmosphere fluxes of the greenhouse gases CO_2 , CH_4 and N_2O in a mountain spruce forest subjected to long-term N addition and to tree girdling. *Agric For Meteorol* 181:61–8.
- Lepš J, Šmilauer P. 2003. Multivariate Analysis of Ecological Data using CANOCO. Cambridge University Press
- Schleppi P, Muller N, Edwards PJ, Bucher JB. 1999. Three years of increased nitrogen deposition do not affect the vegetation of a montane forest ecosystem. *Phyton* 39:197–204.