Eyes on the future – evidence for trade-offs between growth, storage and defense in Norway spruce

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The following **Supporting Information** is available for this article:

Figure S1 Concentrations of soluble sugars, starch and NSC (soluble sugars + starch) expressed as percentage of control (400 ppm [CO_2]) at the whole-tree level.

Figure S2 Concentrations of soluble sugars, starch and NSC (soluble sugars + starch) at the whole-tree level.

Figure S3 Concentrations of phenolic compounds, monoterpenes and total secondary metabolites expressed as percentage of control (400 ppm [CO_2]) at the whole-tree level.

Figure S4 Concentrations of phenolic compounds, monoterpenes and total secondary metabolites (phenolic compounds + monoterpenes) at the whole-tree level.

Figure S5 $\delta^{13}C$ (‰) of bulk tissue, water soluble C, and phenolic compounds at the whole-tree level.

Table S1 Internal standards, weight-based response factors and methods used for the measurements of secondary metabolites.

Table S2 A rough estimation of allocation of newly-assimilated carbon.

Method S1: TD-GC-MS conditions for BOVC analysis

Fig. S1 Concentrations of soluble sugars (a-e), starch (f-j), and total NSC (soluble sugars + starch, k-o) of young needles and branches, old needles and branches and roots in *Picea abies* for the different [CO_2] treatments: 400 ppm [CO_2] (squares, blue line); 400-280-120 ppm [CO_2] (circles, black line); 400-170-50 ppm [CO_2] (triangles, red line), expressed as a percentage of control (400 ppm [CO_2]). The black and red dashed lines indicate reducing [CO_2] from 400 to 280 ppm and from 400 to 170 ppm, respectively, maintained for two weeks; the black and red solid lines indicate a further reduction from 280 to 120 ppm and from 170 to 50 ppm, respectively, maintained for six weeks. Error bars at 400 ppm [CO_2] represent coefficient of variation (*i.e.* relative standard error). Error bars at 280, 170 120 and 50 ppm [CO_2] represent propagated standard errors. Significant differences between the two lower [CO_2] treatments and ambient [CO_2] (400 ppm) are calculated based on the raw concentrations, and indicated by filling of symbols ($P<0.05$, Tukey's HSD). Note that we did not perform statistical test for the old organs at week 8, because of a lack of samples from two spruce trees at 50 ppm [CO_2].

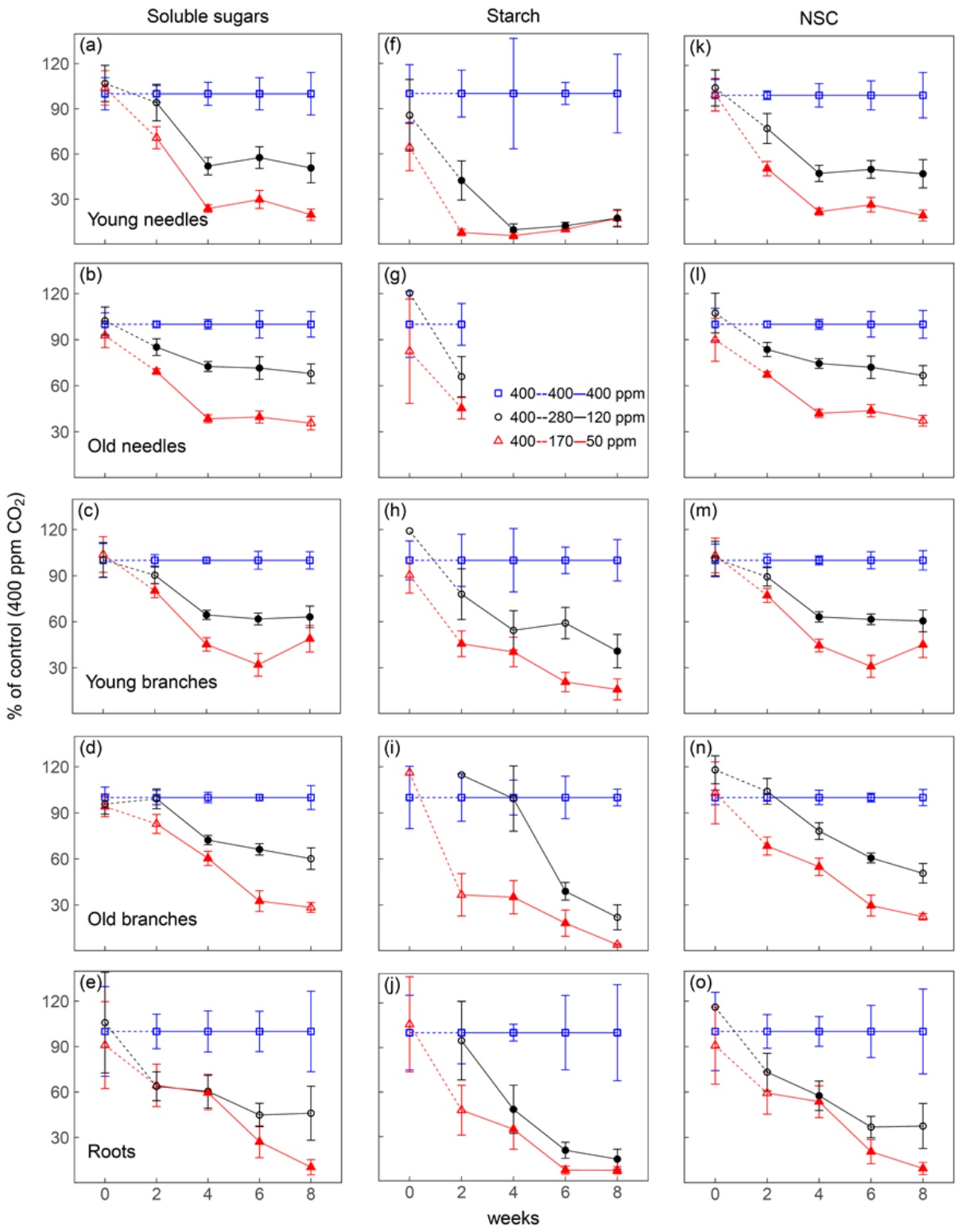
Soluble sugars
Starch
NSC
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
Young needles
Old needles
Young branches
Old branches
Roots
400--400—400 ppm
400--280—120 ppm
400--170—50 ppm
% of control (400 ppm CO2)
weeks
120
90
60
30
0
2
4
6
8

Fig. S2 Concentrations of soluble sugars (mg g^{-1} dry weight, a-e), starch (mg g^{-1} dry weight, f-j), and total NSC (soluble sugars + starch, k-o) of young needles and branches, old needles and branches and roots in *Picea abies* for the different [CO_2] treatments: 400 ppm [CO_2] (squares, blue line); 400-280-120 ppm [CO_2] (circles, black line); 400-170-50 ppm [CO_2] (triangles, red line). The black and red dashed lines indicate reducing [CO_2] from 400 to 280 ppm and from 400 to 170 ppm, respectively, maintained for two weeks; the black and red solid lines indicate a further reduction from 280 to 120 ppm and from 170 to 50 ppm, respectively, maintained for six weeks. Values are the means of four individual chambers; error bars represent ± SE. Significant differences between the two lower [CO_2] treatments and ambient [CO_2] (400 ppm) are indicated by filling of symbols ($P<0.05$, Tukey's HSD). Note that we did not perform statistical test for the old organs at week 8, because of a lack of samples from two spruce trees at 50 ppm [CO_2].

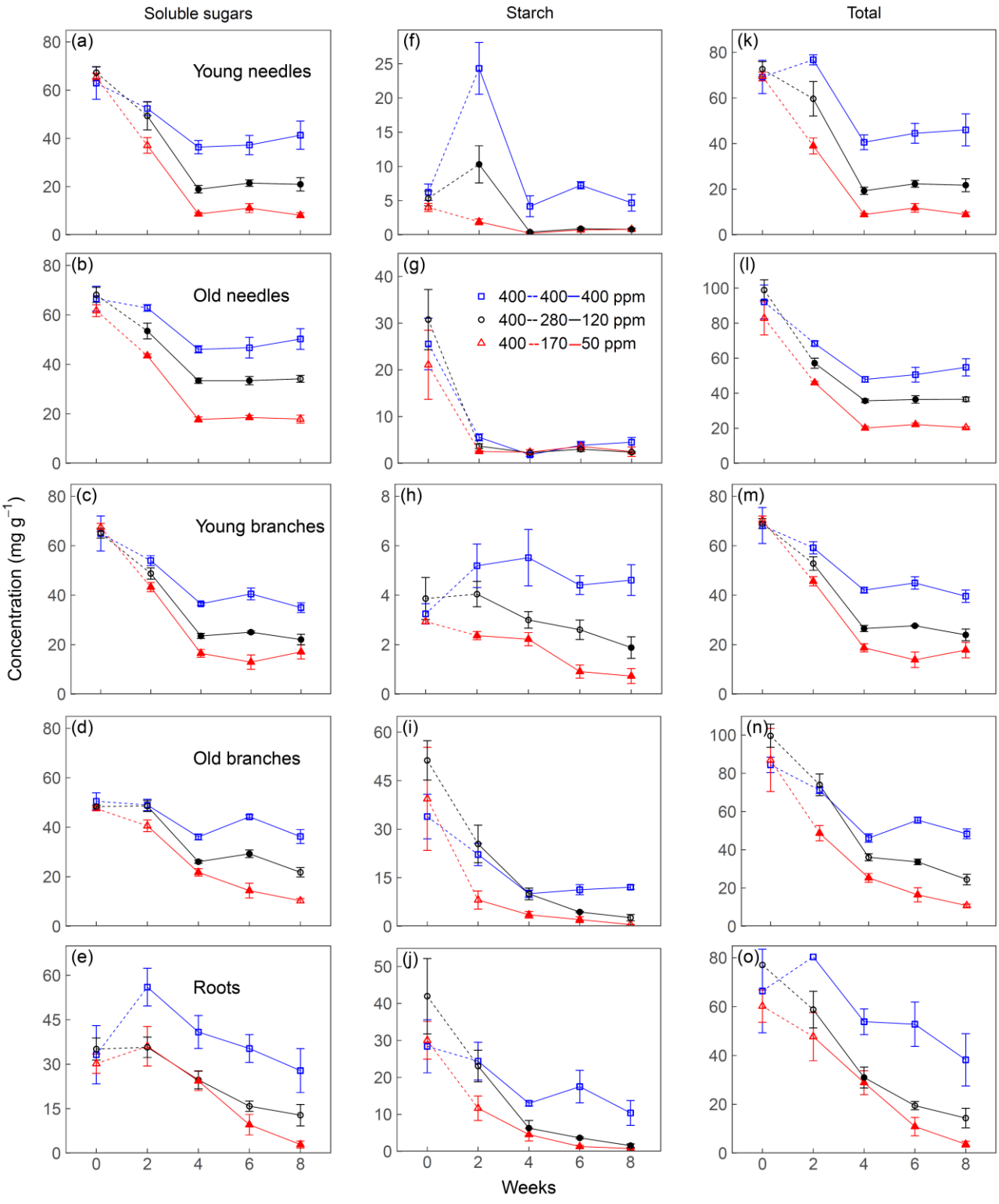
Soluble sugars
Starch
Total
(a)
Young needles
(b)
Old needles
(c)
Young branches
(d)
Old branches
(e)
Roots
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
400--400—400 ppm
400--280—120 ppm
400--170—50 ppm
Concentration (mg g^{-1})
Weeks

Fig. S3 Concentrations of phenolic compounds (a-e), monoterpenes (f-j), and total secondary metabolites (phenolic compounds + monoterpenes, k-o) of young needles and branches, old needles and branches and roots in *Picea abies* for the different [CO_2] treatments: 400 ppm [CO_2] (squares, blue line); 400-280-120 ppm [CO_2] (circles, black line); 400-170-50 ppm [CO_2] (triangles, red line), expressed as a percentage of control (400 ppm [CO_2]). The black and red dashed lines indicate reducing [CO_2] from 400 to 280 ppm and from 400 to 170 ppm, respectively, maintained for two weeks; the black and red solid lines indicate a further reduction from 280 to 120 ppm and from 170 to 50 ppm, respectively, maintained for six weeks. Error bars at 400 ppm [CO_2] represent coefficient of variation (*i.e.* relative standard error). Error bars at 280, 170 120 and 50 ppm [CO_2] represent propagated standard errors. Significant differences between the two lower [CO_2] treatments and ambient [CO_2] (400 ppm) are calculated based on the raw concentrations, and indicated by filling of symbols ($P<0.05$, Tukey's HSD). Note that we did not perform statistical test for the old organs at week 8, because of a lack of samples from two spruce trees at 50 ppm [CO_2].

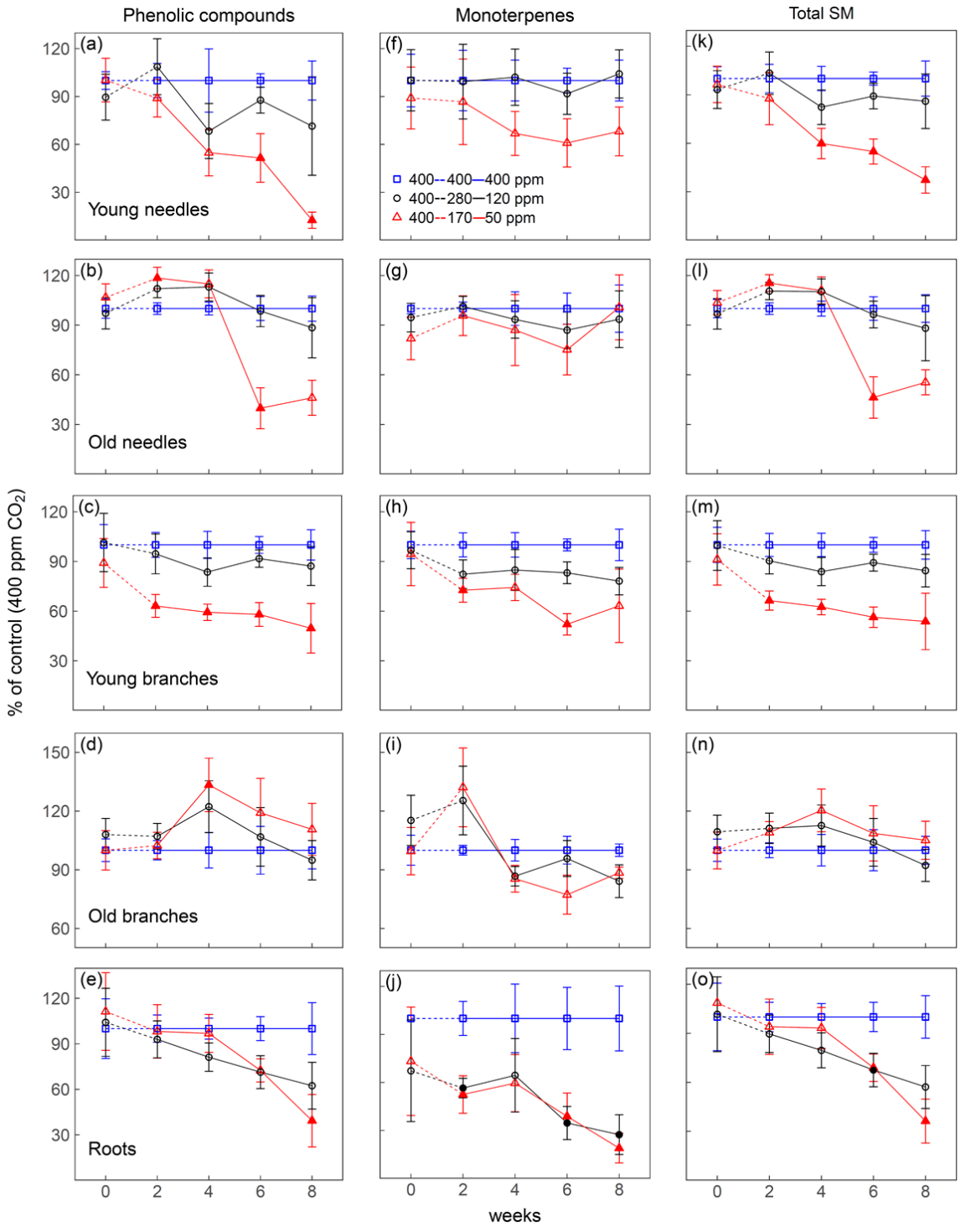
Phenolic compounds
Monoterpenes
Total SM
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
Young needles
Old needles
Young branches
Old branches
Roots
400--400—400 ppm
400--280—120 ppm
400--170—50 ppm
% of control (400 ppm CO2)
weeks

Fig. S4 Concentrations of phenolic compounds (mg g^{-1} dry weight, a-e), monoterpenes (mg g^{-1} dry weight, f-j), and total secondary metabolites (phenolic compounds + monoterpenes, k-o) of young needles and branches, old needles and branches and roots in *Picea abies* for the different [CO_2] treatments: 400 ppm [CO_2] (squares, blue line); 400-280-120 ppm [CO_2] (circles, black line); 400-170-50 ppm [CO_2] (triangles, red line). The black and red dashed lines indicate reducing [CO_2] from 400 to 280 ppm and from 400 to 170 ppm, respectively, maintained for two weeks; the black and red solid lines indicate a further reduction from 280 to 120 ppm and from 170 to 50 ppm, respectively, maintained for six weeks. Values are the means of four individual chambers; error bars represent ± SE. Significant differences between the two lower [CO_2] treatments and ambient [CO_2] (400 ppm) are indicated by filling of symbols ($P<0.05$, Tukey's HSD). Note that we did not perform statistical test for the old organs at week 8, because of a lack of samples from two spruce trees at 50 ppm [CO_2].

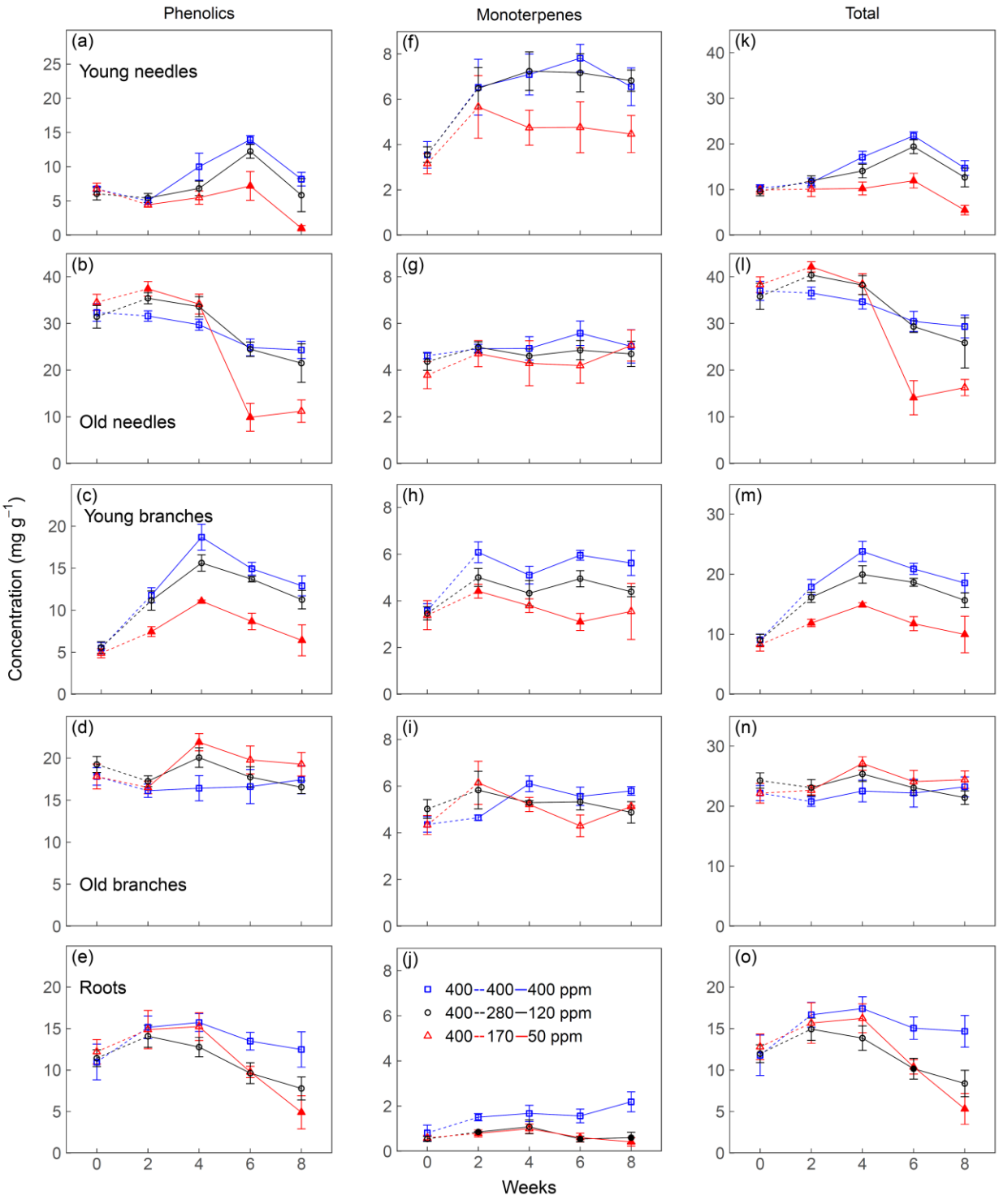
Phenolics
Monoterpenes
Total
(a)
Young needles
(b)
Old needles
(c)
Young branches
(d)
Old branches
(e)
Roots
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
(o)
400--400—400 ppm
400--280—120 ppm
400--170—50 ppm
Concentration (mg g^{-1})
Weeks

Fig. S5 $\delta^{13}C$ (‰) of bulk tissue (a-e), water soluble C (f-j) and phenolic compounds (k-n) in *Picea abies* for the different [CO_2] treatments: 400 ppm [CO_2] (squares, blue line); 400-280-120 ppm [CO_2] (circles, black line); 400-170-50 ppm [CO_2] (triangles, red line). The black and red dashed lines indicate reducing [CO_2] from 400 to 280 ppm and from 400 to 170 ppm, respectively, maintained for two weeks; the black and red solid lines indicate a further reduction from 280 to 120 ppm and from 170 to 50 ppm, respectively, maintained for six weeks. Values are the means of four individual chambers; error bars represent ± SE. Significant differences between the two lower [CO_2] treatments and ambient [CO_2] (400 ppm) are indicated by filling of symbols ($P<0.05$, Tukey's HSD or Wilcoxon's rank-sum test). Note that we did not perform statistical test for the old organs at week 8, because of a lack of samples from two spruce trees at 50 ppm [CO_2].

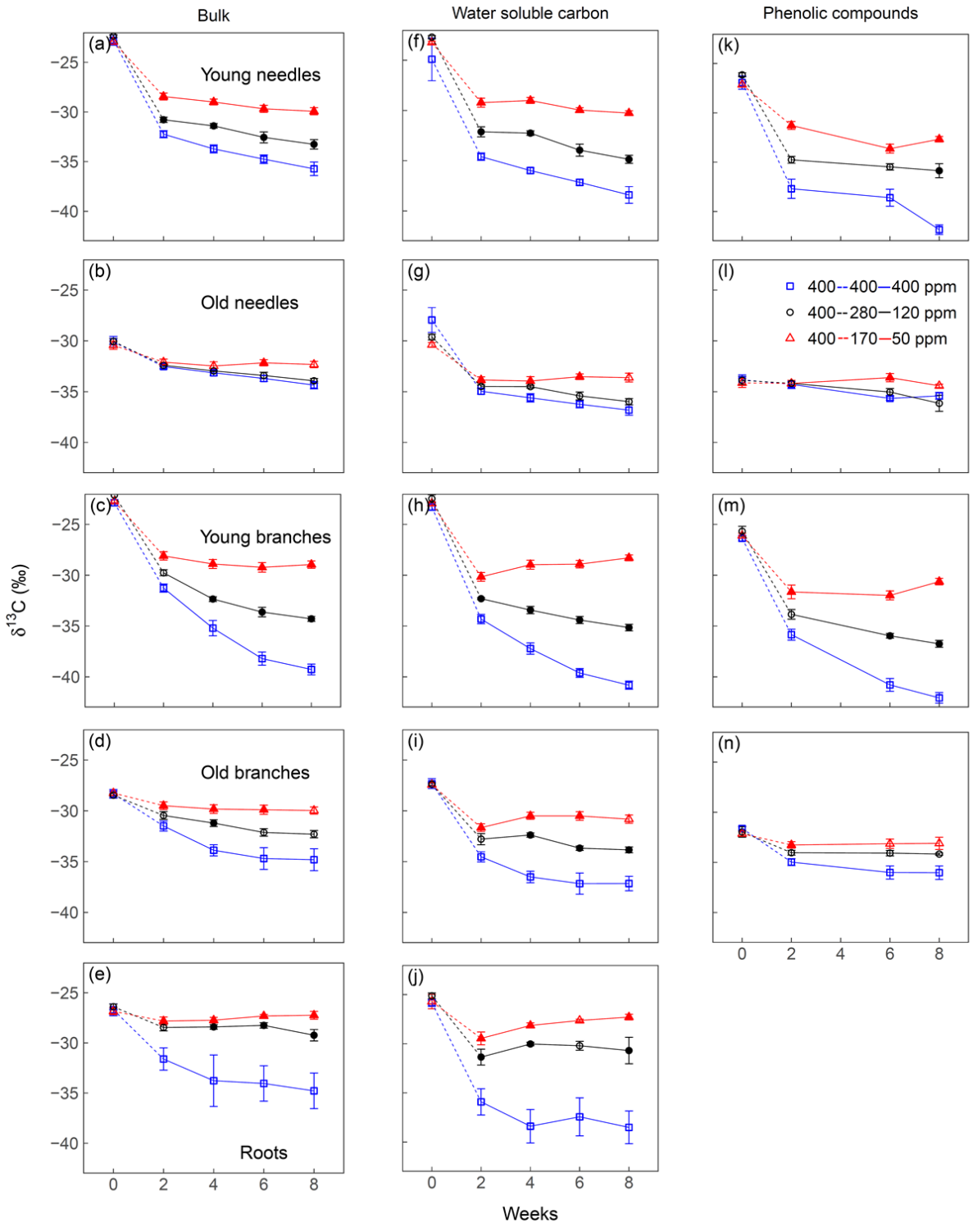
Bulk
Water soluble carbon
Phenolic compounds
(a)
Young needles
(b)
Old needles
(c)
Young branches
(d)
Old branches
(e)
Roots
(f)
(g)
(h)
(i)
(j)
(k)
(l)
(m)
(n)
400--400—400 ppm
400--280—120 ppm
400--170—50 ppm
δ13C (‰)
Weeks
−25
−30
−35
−40
0
2
4
6
8

Table S1 Internal standards, weight-based response factors and methods used for the measurements of secondary metabolites.

Secondary metabolites	Internal Standards	Response factor	Molecular formula	Molecular weight (g mol^{-1})	Method
Catechin	Apigenin-7-glucoside	5.2	$C_{15}H_{14}O_6$	290	LC-MS
Taxifolin	Apigenin-7-glucoside	1.1	$C_{15}H_{12}O_7$	304	LC-MS
Gallocatechin	Apigenin-7-glucoside	5.2	$C_{15}H_{14}O_7$	306	LC-MS
Astringin	Apigenin-7-glucoside	0.9	$C_{20}H_{22}O_9$	406	LC-MS
Isorhapontin	Apigenin-7-glucoside	3.5	$C_{21}H_{24}O_9$	420	LC-MS
Proanthocyanidin B1	Apigenin-7-glucoside	10.1	$C_{30}H_{26}O_{12}$	578	LC-MS
α-pinene	1,9-decadiene	0.97	$C_{10}H_{16}$	136	GC-FID
camphene	1,9-decadiene	0.97	$C_{10}H_{16}$	136	GC-FID
β-pinene	1,9-decadiene	0.97	$C_{10}H_{16}$	136	GC-FID
Myrcene	1,9-decadiene	0.97	$C_{10}H_{16}$	136	GC-FID
Limonene	1,9-decadiene	0.97	$C_{10}H_{16}$	136	GC-FID
1, 8-cineole	1,9-decadiene	1.09	$C_{10}H_{18}O$	154	GC-FID
Bornyl acetate	1,9-decadiene	1.27	$C_{12}H_{20}O_2$	196	GC-FID

Table S2 A rough estimation of the percentage of aboveground cumulative assimilation (ACA) allocated to aboveground cumulative respiration (ACR), non-structural carbohydrates (NSC), secondary metabolites (SM) and Unaccounted C pool (UCP). Concentrations of NSC and SM (mg g^{-1} FW) were scaled to whole-tree level by multiplying with estimated biomass fractions of each of needles, branches and roots. Changes in NSC, SM and unaccounted carbon pool (including growth, root respiration and exudation; expressed as changes in carbon content) were then calculated using the following equations: UCP = ACA – ACR – ΔNSC – ΔSM; $\Delta NSC = (FW_{week6} * NSC_{week6} - FW_{week0} * NSC_{week0}) * 0.4$; $\Delta SM = (FW_{week6} * SM_{week6} - FW_{week0} * SM_{week0}) * 0.4$.

Treatment	*ACC (%)*	*ACR (%)*	*NSC (%)*	*SM (%)*	*UCP (%)*
400 ppm	*100*	*19*	*4*	*4*	*73*
400-280-120 ppm	*100*	*31*	*-20*	*3*	*86*
400-170-50 ppm	*100*	*80*	*-118*	*5*	*133*

Methods S1 TD-GC-MS conditions for BOVC analysis

BVOC Tubes were desorbed with helium as carrier gas and a flow path temperature of 150 ^{o}C using the following conditions: dry purge for 5 min at 20 ml/min, pre purge for 2 min at 20 ml/min, desorption for 8 min at 280 ^{o}C with 20 ml/min, pre trap fire purge for 1 min at 30 ml/min, trap heated to 300 ^{o}C and hold for 4 min. The BVOC were separated on a GC (Bruker, GC-456, Bremen, Germany) connected to a triple-quad MS (Bruker, SCION). Separation took place on a DB-5MS column (30 m x 0.25 mm x 0.25 µm. Restek, Germany). The oven was programmed from an initial temperature of 40°C (6-min hold), followed by an increase to 120°C at 20°C min^{-1} and to 200 °C at 5°C min^{-1}, and then to 260 °C at 30°C min^{-1} and hold at 260 °C for 10 min. The MS was operated as follows: full scan from 40 to 550 m/z; electron energy, 70eV; transfer line temperature, 260°C; ion source temperature, 240°C; manifold 40°C.