

# **Different response of mistletoe and Scots pine in Pfynewald**

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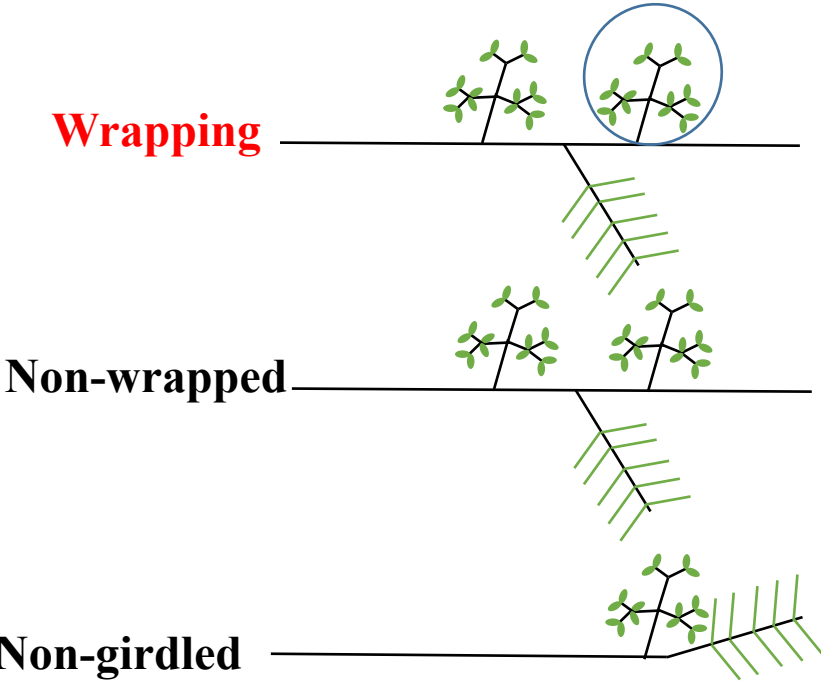
# Background

- **No study has actually shown that mistletoe could directly absorb carbon resources from host tissues. There is only some indirect evidence, based on comparable seasonal variations of sugar concentrations in the host and mistletoe xylem.**
- **Few studies have focused on the nutrient and carbon uptake by mistletoes from the host and the different absorption rates under varying environmental condition (i.e. soil drought and sufficient soil water), also the dynamics and the controlling factors of this relationship between mistletoe and host are still unclear.**

# Experiment Design



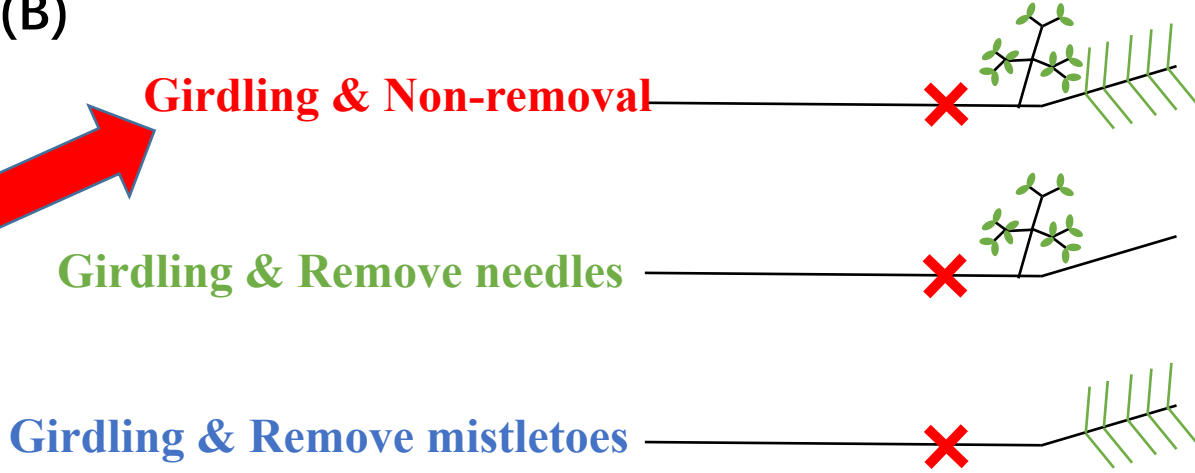
(A)



Wrapping experiment



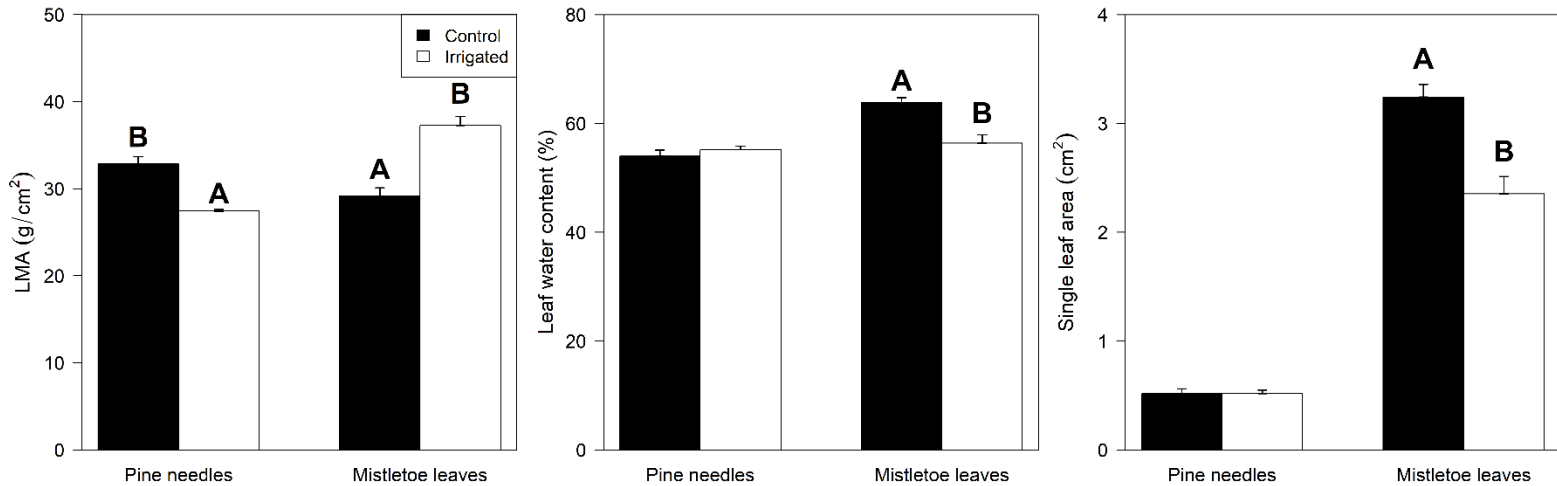
(B)



Girdling and Removal experiment

# Hypothesis

- **1) Mistletoes can photosynthesis itself but not directly take up carbon assimilates directly from the host tree, and the photosynthesis rate will be higher in irrigated trees comparing to the non-irrigated trees;**
- **2) Mistletoes will benefit and take up more carbon resource when transportation from the needles to other tissues was blocked (girdling vs non- girdled control), especially in the relative stressed condition (non-irrigated vs irrigated);**
- **3) If, however, host source activity is impaired (girdling + needle removal), mistletoes could perform as a source tissues and transfer carbon assimilates to sink tissues of the host, which will be more conspicuous when the host tress grown in relative drought stressed conditions**

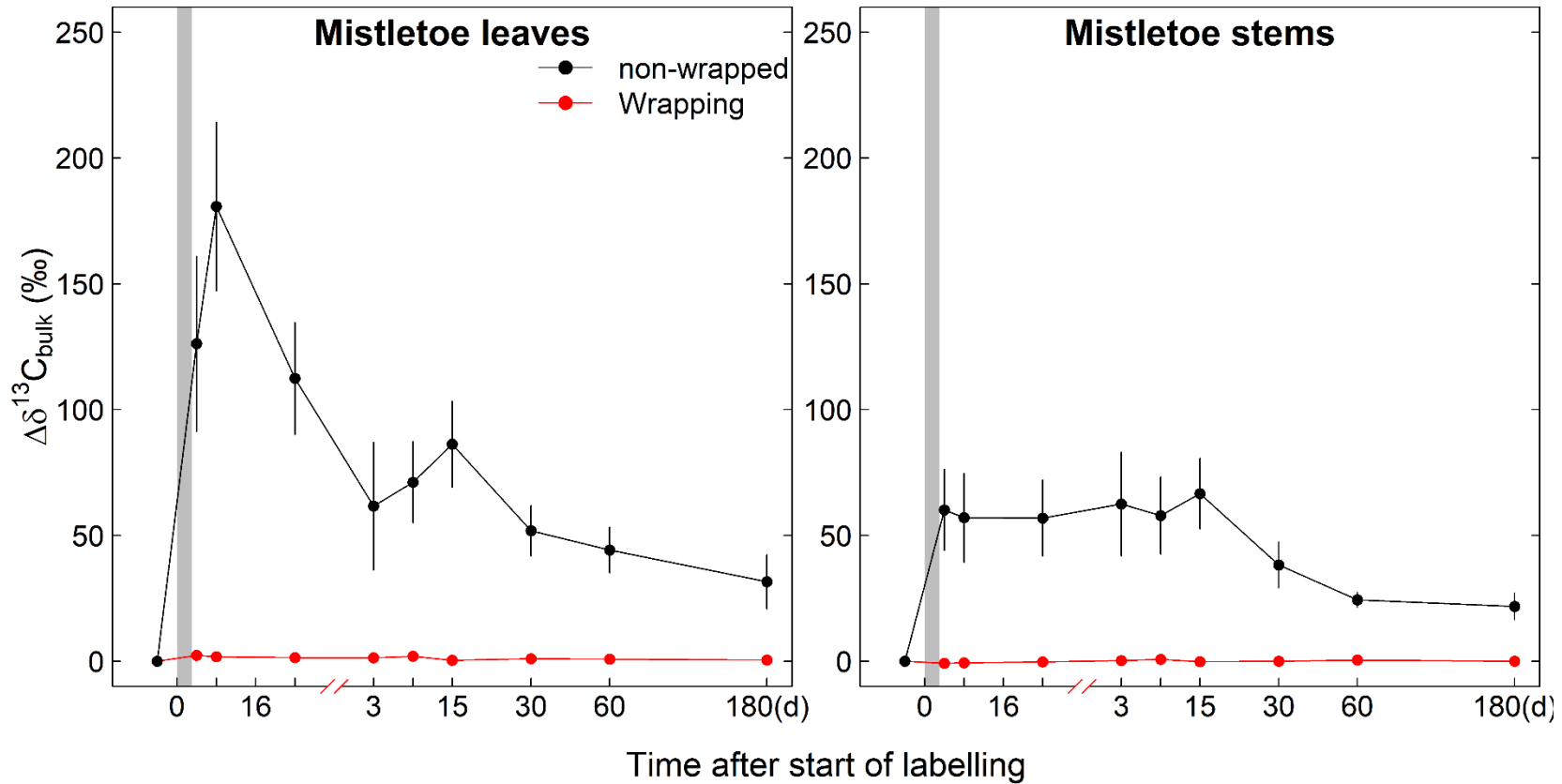


**Figure 2** Comparison of the leaf traits between *Pinus Sylvestris* and *Viscum album ssp. Austriacum*. (a) Leaf mass per area (LMA) of mistletoe leaves and pine needles in different irrigation treatment. (b) Leaf water content of mistletoe leaves and pine needles in different soil moisture treatment. (c) Single leaf area of mistletoe leaves and pine needles in different soil moisture treatment.

**Leaf mass per area (LMA) was significant higher in control pine needles than in the irrigated ones, but the mistletoe leaves showed the opposite pattern**

**Leaf water content was higher in control compared to irrigated mistletoe leaves.**

**Mistletoe had bigger leaves under dry control conditions.**



**Figure 3:** Incorporation of  $^{13}\text{C}$ -label into assimilates for mistletoe leaves and stems in different wrapping treatment (i.e. wrapped/ not) after exposure to a 4 h  $^{13}\text{C}$ -enriched  $\text{CO}_2$  labelling event (shaded area) and during the period. Please note the initial point (one day before the labelling) and scale of the x-axis variation (n=3 per individual treatment).

**Strong labelling effect was found on the non-wrapped mistletoe leaves and stems but not in wrapping ones.**

**The peak value occurred after around 8 hours after labelling, which had an approximately 4 hours lag after the labelling.**

**The  $^{13}\text{C}$  values reached the peak value in mistletoe stems as soon as the labelling event finished,**

**Table 1:** Results of linear mixed models for  $\Delta\delta^{13}\text{C}$  values (uptake and incorporation of  $^{13}\text{C}$  of bulk material in different tissues of *Pinus Sylvestris* and *Viscum album ssp. Austriacum* of the girdling and removal experiment.

Girdling Experiment			
Tissue	Factors	df	$\Delta\delta^{13}\text{C}$
Needle	Time	6	<0.001***
	Irrigation	1	0.88
	Girdling	1	0.12
Phloem	Time	6	0.002**
	Irrigation	1	0.44
	Girdling	1	0.20
Xylem	Time	6	0.20
	Irrigation	1	0.72
	Girdling	1	0.35
Mistletoe leaf	Time	6	<0.001***
	Irrigation	1	0.07
	Girdling	1	0.51
Removal Experiment			
Tissue	Factors	df	$\Delta\delta^{13}\text{C}$
Needle	Time	6	<0.001***
	Irrigation	1	0.77
	Removal	1	<0.001***
Phloem	Time	6	<0.001***
	Irrigation	1	0.69
	Removal	2	0.002**
Xylem	Time	6	0.01**
	Irrigation	1	0.73
	Removal	2	<0.001***
Mistletoe leaf	Time	6	<0.001***
	Irrigation	1	0.03*
	Needle Removal	1	<0.001***

\*p<0.05, \*\*p<0.01, \*\*\*p<0.001

**Irrigation treatment had no effect on  $^{13}\text{C}$  incorporation and nitrogen concentrations in all pine tissues.**

**Either mistletoe removal or needle removal affected the  $^{13}\text{C}$  incorporation in tissues.**

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**Table 2:** Results of linear mixed models for concentration of  $\Delta$ NSC (the increase percentage of non-structural carbohydrate during the period) and its compounds (i.e.  $\Delta$ sugar &  $\Delta$ starch) in two sub-experiments which based on the  $^{13}\text{C}$  labelling experiment.

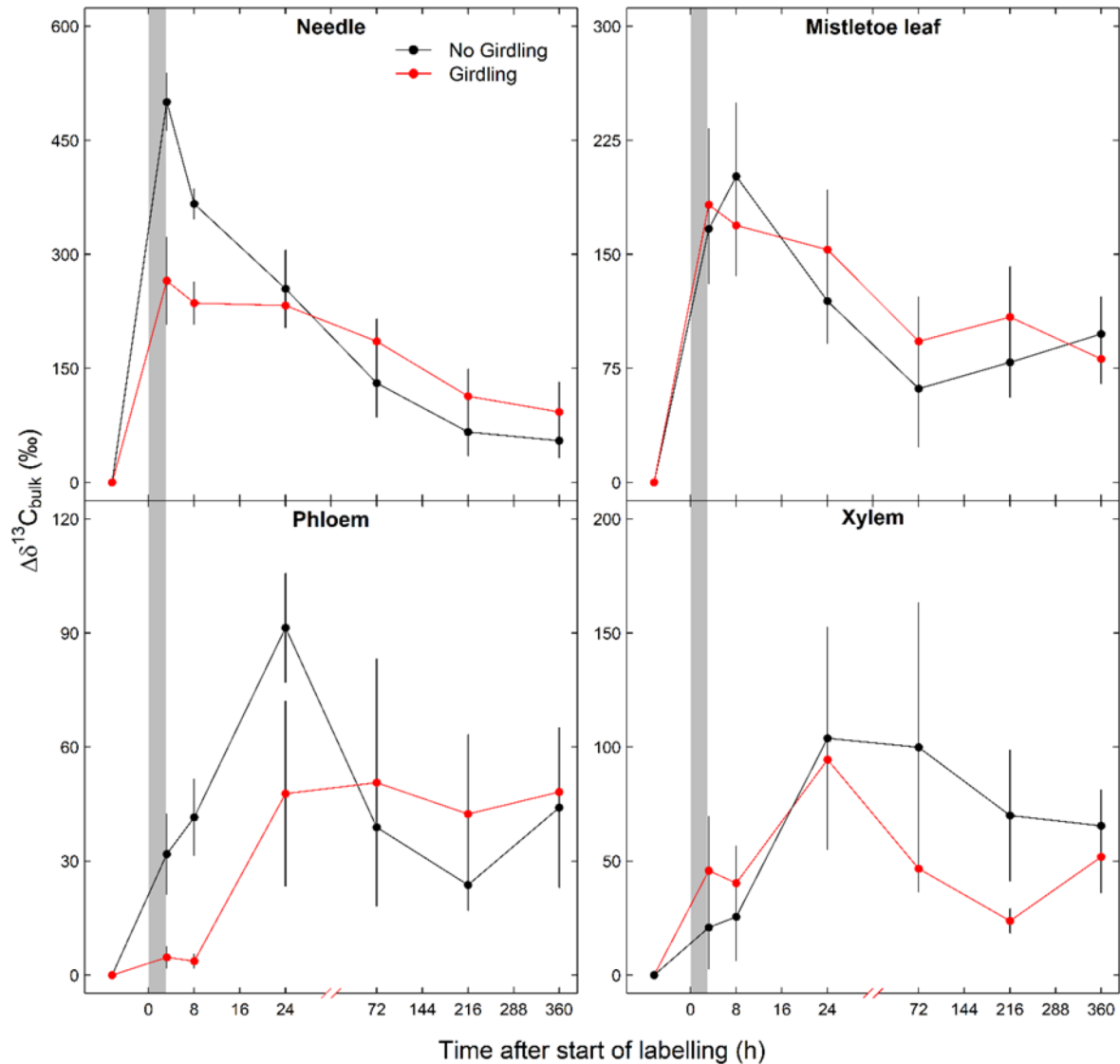
Girdling Experiment					
Tissue	Factors	df	$\Delta$ NSC	$\Delta$ Sugar	$\Delta$ Starch
Needle	Time	4	0.49	0.57	0.32
	Irrigation	1	0.45	0.93	0.23
	Girdling	1	0.008**	0.04*	0.02*
Phloem	Time	4	0.41	0.02**	<0.001***
	Irrigation	1	0.46	0.82	0.47
	Girdling	1	<0.001***	<0.001***	0.03*
Xylem	Time	4	0.38	0.04*	0.96
	Irrigation	1	0.60	0.72	0.37
	Girdling	1	0.14	0.03*	0.95
Mistletoe leaf	Time	4	0.18	0.03*	0.25
	Irrigation	1	0.16	0.62	0.16
	Girdling	1	0.28	0.12	0.57
Removal Experiment					
Tissue	Factors	df	$\Delta$ NSC	$\Delta$ Sugar	$\Delta$ Starch
Needle	Time	4	0.59	0.98	0.60
	Irrigation	1	0.88	0.82	0.75
	Mistletoe Removal	1	0.05*	0.76	0.05*
Phloem	Time	4	0.88	0.08	0.17
	Irrigation	1	0.29	0.95	0.16
	Removal	2	0.008**	0.25	0.05*
Xylem	Time	4	0.02*	0.06	0.46
	Irrigation	1	0.15	0.09	0.46
	Removal	2	<0.001***	<0.001***	0.11
Mistletoe leaf	Time	4	0.56	0.01**	0.84
	Irrigation	1	0.03*	0.20	0.04*
	Needle Removal	1	0.40	0.27	0.19

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

**For girdling experiment, NSC concentrations were not affected by irrigation treatments in pine tissues.**

**For removal experiment, irrigation treatments did not affect pine tissues. However, NSC in mistletoe leaves were affected by different irrigation treatments. Meanwhile, removal treatments had effects on pine tissues but not for mistletoe leaves.**

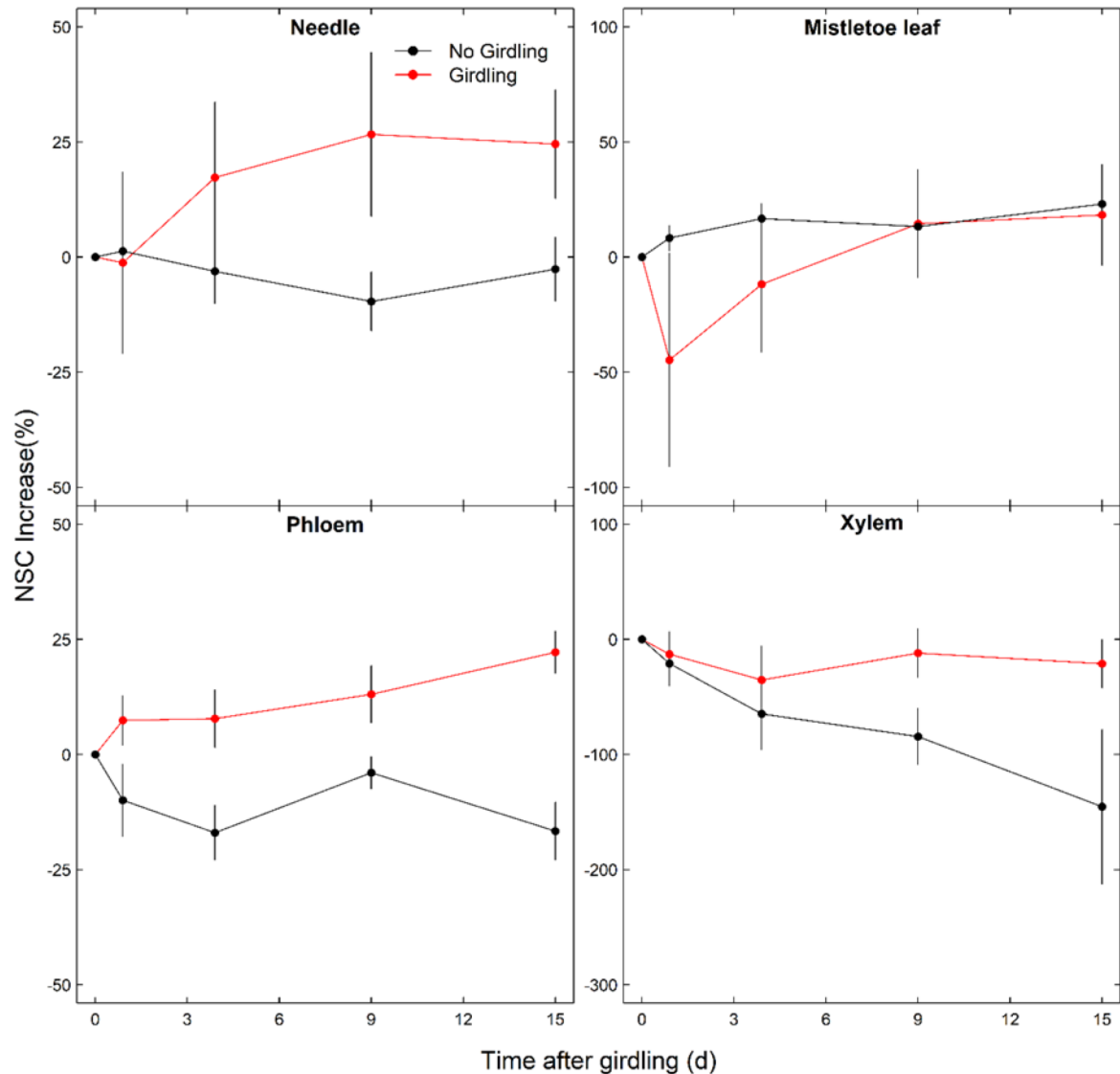




**Figure 4:** Girdling effect (one day before the labelling, i.e. initial point) on Incorporation of  $^{13}\text{C}$ -label into assimilates ( $\Delta\delta^{13}\text{C}$  values) of bulk ( $\Delta\delta^{13}\text{C}_{\text{bulk}}$ ) in different pine (*Pinus Sylvestris*) and mistletoe (*Viscum album ssp. Austriacum*) tissues after exposure to a 4 h  $^{13}\text{C}$ -enriched  $\text{CO}_2$  labelling event (shaded area).

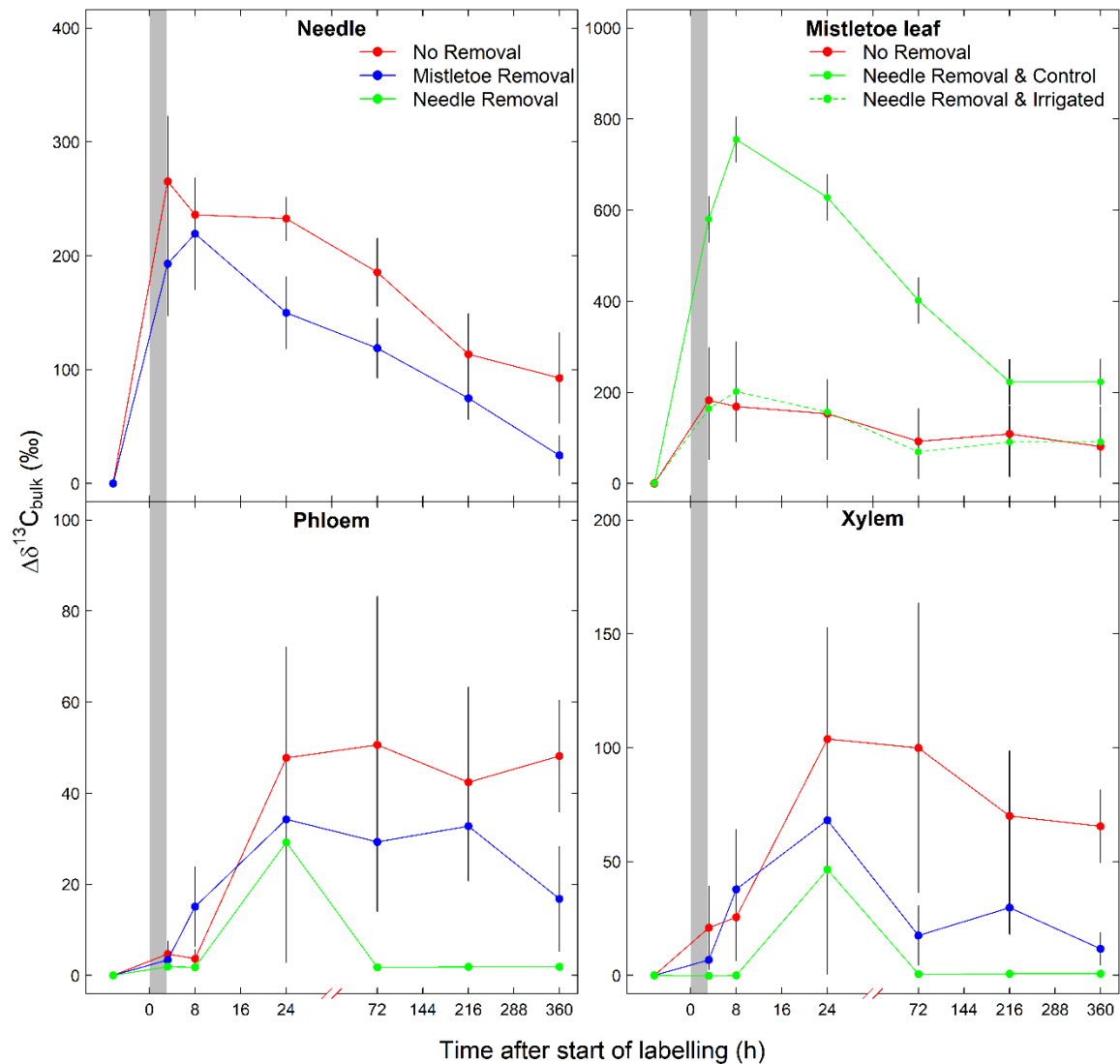
**Girdling treatment significantly decrease the peak value of  $^{13}\text{C}$  incorporation in needles and phloem during the labelling period. The incorporation of  $^{13}\text{C}$  in sink tissues (i.e. phloem & xylem) had an approximately 20 hours lag effect compared to the needles under the girdling treatment.**

**Girdling treatment did not affect the  $^{13}\text{C}$  uptake and nutrient allocation of mistletoe leaves during the labelling period.**



**NSC concentration in needles significantly increased after girdling. However, NSC in mistletoe leaves were not affected by girdling treatment.**

**Figure 5:** Girdling effect (one day before the labelling, i.e. initial point) on the increase of non-structural carbohydrate (%) in different pine (*Pinus Sylvestris*) and mistletoe (*Viscum album ssp. Austriacum*) tissues during the period. Please note the scale of the x-axis variation and y-axis change between upper and lower panel (n=3 per individual treatment).

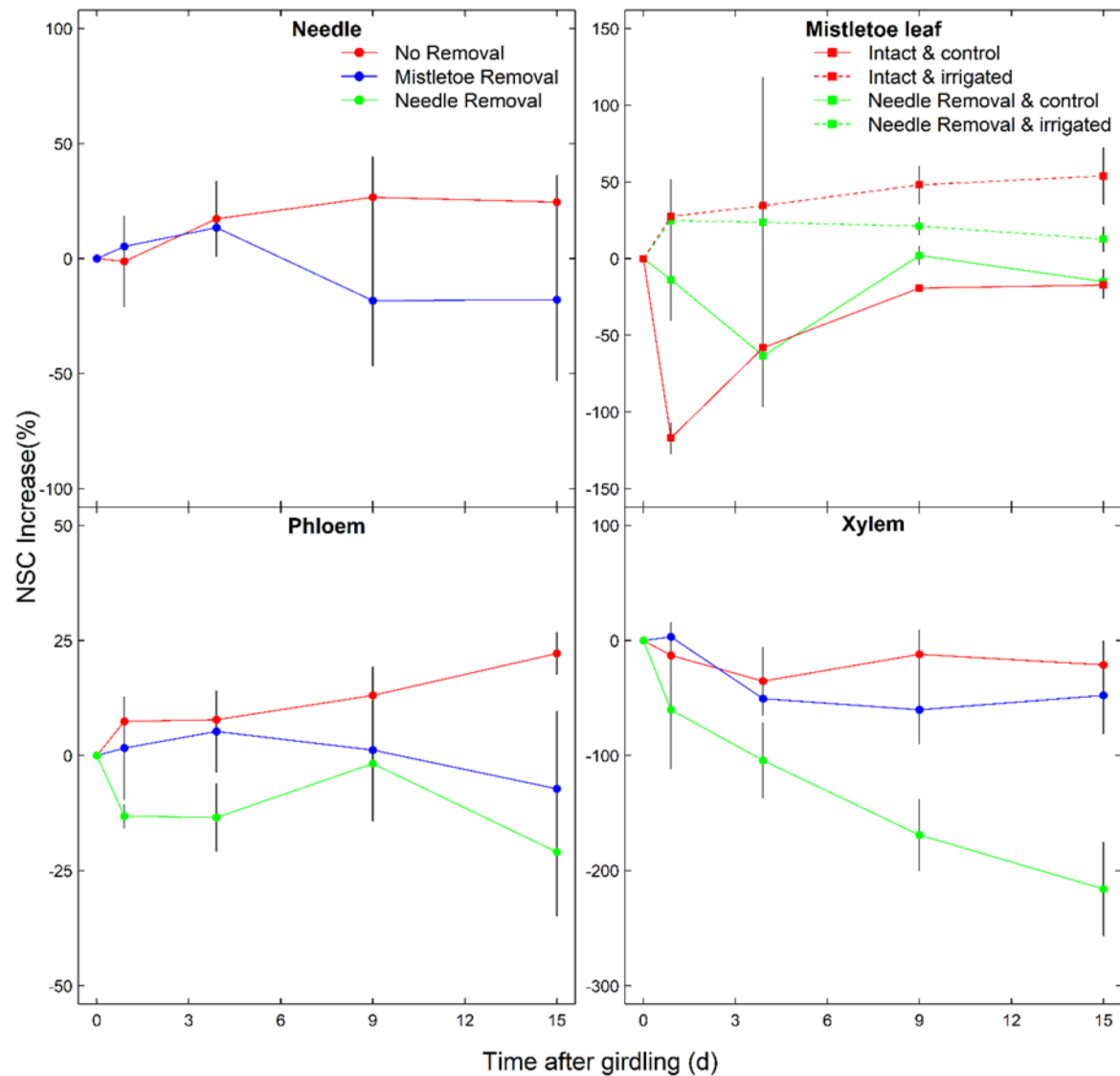


**Mistletoe leaves showed a higher  $^{13}\text{C}$  incorporation in the non-irrigated treatment, when the needles were removed from the branches.**

**Removing the needles from the girdled branches significantly decreased the  $^{13}\text{C}$  incorporation in the branch sink tissues of pine (i.e. phloem & xylem).**

**When mistletoe leaves were removed from the branches,  $^{13}\text{C}$  incorporation decreased in all pine tissues.**

**Figure 6:** Incorporation of  $^{13}\text{C}$ -label into assimilates ( $\Delta\delta^{13}\text{C}$  values) of bulk ( $\Delta\delta^{13}\text{C}_{\text{bulk}}$ ) in different pine (*Pinus Sylvestris*) and mistletoe (*Viscum album ssp. Austriacum*) tissues under different removal treatments (i.e. needle removal/ mistletoe removal) and exposure to a 4 h  $^{13}\text{C}$ -enriched  $\text{CO}_2$  labelling event (shaded area) on girdling (one day before the labelling, i.e. initial point) branches.



**For mistletoe leaves, NSC concentration significantly decreased in the non-irrigated treatment compared to irrigated treatment, independent if pine needles were removed or not.**

**Either pine needles or mistletoe leaves removal decreased the concentration of NSC in the pine branch sink tissues over the sampling period. The same occurred in pine needles.**

**Figure 7:** Increase of non-structural carbohydrate (%) in different pine (*Pinus Sylvestris*) and mistletoe (*Viscum album ssp. Austriacum*) tissues under different removal treatments (i.e. needle removal/ mistletoe removal) on girdling (one day before the labelling, i.e. initial point) branches.

# Conclusion

- 1. No  $^{13}\text{C}$  signal in the wrapping mistletoe leaves, indicating that there was no C-transfer from the host to the mistletoes via the phloem sap. Lower carbon assimilates in mistletoes compared with the hosts can be included by the high transpiration and low gross photosynthesis rate of mistletoes.**
- 2. Girdling treatment could result in a higher NSC concentrations and carbon in pine tissues, but not for mistletoe leaves, indicating a kind of negative feedback on photosynthesis because of the carbon transportation was cut off.**
- 3. When needles were completely removed from the girdled branches, host xylem and phloem were still able to acquire  $^{13}\text{C}$  from the mistletoes. Those results suggest that mistletoes can support the host with carbon resources, especially when the host is C resource limited (needle removal + girdling). Mistletoes may play a role as C source providers to support the host and maintain a symbiotic relationship to survive.**

# Thanks for listening !

