Temporary title: **Effects of soil cooling on *Betula pendula* source activity** / photosynthetic gas exchange

**Introduction**

1. ~~Source and sink; phloem; Munch theory (ch 10 + other books).~~ ~~Betula characteristics (habitat, corologia), esp when it comes to xyl and phl.~~ OK
2. ~~Introduce thesis topic (how does sink affect source? What happens if we change conditions at sink level?)~~ + Papers about similar topics, on other species (Yann articles, google sch, web of science) – used Yann articles (girdling, defruiting, sugar sensing, genetics) + 3 of my articles OK
3. Water potential and stomatal conductance/ evapotranspiration – explain stomata as well

~~Thesis aim: Xin’s PhD (abstract) – to contextualize~~

Other ideas:

Connection to ecosystems? (Not really)

Role of water potential and gas exchange as stress parameters for water status (article) + una parte sulla fotosintesi? + spiega cosa sono parametri gsw (what does that stand for?) e A/Ci (Photosynthetic efficiency – how much carbon you assimilate for amount of carbon inside the leaf. Ci is concentration in the leaf!). articoli su water stress parameters

Gsw – stomi chiusi = stomatal conductance va giù. Pianta stressata non può fare tanta fotosintesi perché non può entrare co2. Collegata a evapotraspiration perché chiaramente se stomi sono chiusi, evapora meno acqua 🡪 se ne va giù una va giù anche l’altra, ed entrambe sono collegate all’efficienza della fotosintesi.

Soil temperature effect (article)

(Sap flow velocity and labelling could be put in discussion (under “further analysis”)?). so I could move that paragraph from mat and meth to discussion.

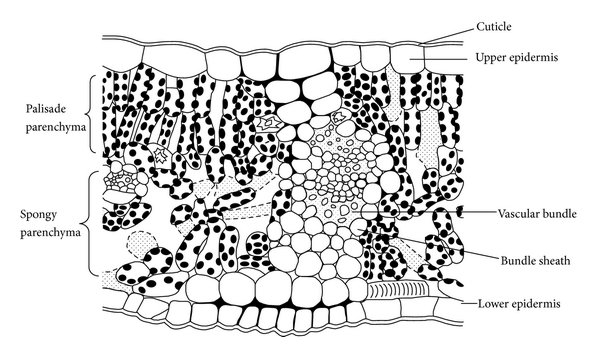
1. Photosynthesis
2. Water potential and stomata
3. Phloem transport
4. Betula pendula

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Photosynthesis is the basis of the food chain and “the ultimate source of all of humankind’s food and oxygen” (Johnson, 2016). Oxygenic photosynthesis is a process that allows certain organisms, including plants, to harvest solar energy to synthesize carbon compounds, starting from carbon dioxide and water and generating oxygen as well (Taiz & Zeiger, ):

6 CO2 + 6 H2O → C6H12O6 + 6 O2

In plants, photosynthesis mostly takes place in the leaves. Figure … shows the typical anatomy of a leaf. Different cell types in the leaf contribute to the photosynthetic process: CO2 entry is controlled by stomata in the lower epidermis, photosynthetic parenchyma (mesophyll) cells contain many chloroplasts and are the principal center of photosynthesis, whereas vascular tissues transport the sap with the necessary compounds into the leaves (xylem), or towards the rest of the plant (phloem) (Mauseth J., 2006).



Typical leaf anatomy. A leaf contains a waxy cuticle, epidermal cells on its upper and lower surface, two types of mesophyll (palisade parenchyma and spongy parenchyma) and transport tissues (xylem and phloem) which are present in the vascular bundle. Figure from Diah et al., 2014.

The site of photosynthesis in the cell is the chloroplast, an organelle with two outer membranes and one thylakoid membrane. The latter divides the chloroplast into two spaces: the stroma on the outside and the lumen on the inside.

In plants, photosynthesis can be divided into two phases:

\start{itemize}

\item A first phase that depends on light and happens in the thylakoid membrane. It results in the production of ATP (high-energy compound) and NAD(P)H (high reducing power). The energy of light is thus converted into chemical energy.

\item A second metabolic phase that happens in the stroma. Here, ATP and NAD(P)H are used by the enzymes of the Calvin-Benson cycle and CO2 gets reduced and fixed into carbohydrates (Taiz & Zeiger, 2002 + Johnson, 2016). ATP and NADPH provide 479 kJ that are necessary for photosynthesis to occur, as it is an endergonic process (Antal et al., 2013).

\end{itemize}

Chloroplast image with explanation of thylakoid, etc.

The first phase of photosynthesis requires pigments, which in plant cells are mainly chlorophyll a and b, i. e. molecules with a ring structure similar to porphyrin (Quieroz et al., 2017). When chlorophyll absorbs photons at certain wavelengths - mostly the blue and red parts of the visible spectrum -, it reaches an excited, unstable state {that cannot be maintained for long}. As the excited molecule goes back to its ground state, the extra energy can be disposed of through energy transfer to a neighboring molecule, which in turn becomes excited (Rascio et al. 2017). This is the process that happens in photosystems I and II, two complexes that can be found in the thylakoid membrane. They are formed by hundreds of pigment molecules that collect and transfer energy to a reaction center, called P680 in photosystem I and P700 in Photosystem II. Two chlorophylls in the reaction center behave differently than other pigments in the complex: instead of transferring energy, one of them undergoes oxidation, transferring an electron to the other and thus making a redox chain possible (Johnson, 2016).

The electron transport chain involves a series of molecules that are present in the thylakoid membrane, ending with ferredoxin/ NADP+ reductase (FNR). This pathway is unidirectional, as electrons are transferred from stronger reductants to weaker ones, except for PSII and PSI, where light energy is required (Johnson, 2016). As a result of the electron chain, NAPD+ is reduced to NADPH, whereas water reduces oxidized P680 back to its former state and is therefore converted to oxygen. The electron chain also causes proton transport across the membrane, with an accumulation of protons in the lumen. This gradient is used by ATP synthase to generate ATP through phosphorylation. (Hill and Bendall, 1960 + Arnon, 1971).

After the light-dependent reactions, there is a metabolic phase: CO2 is fixed into organic compounds during the Calvin-Benson cycle, which is made up of 13 reactions. Primary $CO\_2$ assimilation is catalyzed by the enzyme RuBisCO (ribulose-1,5-bisphosphate carboxylase/ oxygenase), when the sugar ribulose-1,5-bisphosphate undergoes carboxylation. The first three reactions lead to the formation of two molecules of a triose phosphate called glyceraldehyde-3-phosphate (G3P), thanks to the previously produced ATP and NADPH, which provide energy and reducing power. The other ten reactions aim at regenerating RuBP so that it can be used in the next cycle (Biel & Fomina, 2015). In total, the cycle uses 3 molecules of ATP and 2 of NADPH to assimilate one $CO\_2$ molecule (Rascio, 2017).

When three $CO\_2$ and three RuBP molecules generate six G3P molecules, five of these molecules become a part of the cycle again, by being regenerated into RuBP; the sixth molecule is the product that is either converted to starch or transported outside of the chloroplast and then converted to sucrose (Johnson, 2016).

As a whole, photosynthesis is an endergonic process, needing 479 kJ to occur.

**Stomata and water potential**

In 1958, researcher Ralph Slatyer defined water potential as “the energy that is necessary, per unit of volume, to move water […] from an initial point to a reference point”. This concept can be expressed with equation \ref{}:

\psi\_{H\_{2}O} = \mu\_{H\_2O}/V\_{H\_2O},

where \mu is the chemical potential of water (Rascio, 2017).

Water potential is made up of different components, which can be summarized with another equation (\ref{}):

\psi\_{H\_2O} = \psi\_s + \psi\_p + \psi\_g

Leaving out the gravity component ($\psi\_g$), which has a minor role, $\psi\_s$ and $\psi\_p$ have the largest effect on water potential. $\psi\_p$ is hydrostatic pressure. psi{$H\_2O$} gets higher when $\psi\_p$ gets higher. On the contrary, as the osmotic potential ($\psi\_s$) gets higher and solutes accumulate in a plant cell, the water potential gets lower. The presence of a water potential gradient causes water to move from higher to lower values (Taiz & Zeiger, 2002). This concept is fundamental to explain sap transport inside the plant, which is made possible by a pressure gradient ($\delta\psi\_p$) (Rascio, 2017).

Sap (water and minerals) is absorbed by the roots and then travels inside a vascular tissue, the xylem, throughout the root system, the stem and the leaves. (xylem) In the root system, water goes through the exodermis and can then take different pathways, moving through the apoplast, or through the symplast, or across membranes (Steudle, 2000). The roots can contribute directly to this absorption by generating a ($\delta\psi\_{H\_2O}$) between soil and root, which is caused by an osmotic gradient. As ions accumulate in the xylem, $\psi\_s$ increases, and $\psi\_{H\_2O}$ decreases, allowing water to flow in (Gambetta et al., 2017) .

The main force that drives sap transport, however, is a pressure gradient (($\delta\psi\_p$) between leaves and roots, as the cohesion-tension theory claims. According to this theory, water in the xylem behaves like a cohesive, unbroken column that can ascend from roots to leaves because it is driven by evapotranspiration. Transpiration happens inside pores of leaf cell walls. The concavity of the water-air interfaces inside the pores increases as water transpires. Because of capillarity, which aims at restoring the equilibrium, water is pulled against gravity and negative pressure, i. e. tension, is generated. This decrease in hydrostatic potential and water potential is then transmitted to neighboring areas, and eventually to the whole xylem and the roots (Brown, 2013).

*~~Aggiungi parte su xilema. Aggiungi parte della conduttività, prima della parte sul pot idrico.~~ Poi spiega w status.*

*Cose da spiegare:*

*W pot come parametro per status pianta*

*~~Hydraulic conductance (quando spiego le radici)~~ – using wat pot as a proxy for hyd cond – qual è il collegamento hydraulic conductance/ soil cooling?*

*Root cooling: consequences on growth, sink activity, conductance*

*Scholander chamber: Rascio, ma anche Zimmermann*

*How they work (open/ closed) – stomata can close due to less photosynthesis happening (because of sink feedback), or due to lower w pot and a stressed plant. Stomatal conductance and evapotranspiration.*

Significance for plant status! The LWP is a good determinant of leaf water status. The more negative (lower) value of LWP of a plant indicates a more dehydrated leaf (water stressed). Plant-based water stress indicators are quite effective in determining the water stress level in plants because they take into account the cumulative effect of water stress due to declining soil moisture status and increased evaporative demand of the atmosphere while determining the water stress level in plant. Stomatal conductance is a measure of the degree of physical resistance to gas movement between air and leaf interior. Parkash

“stomata control H2O vapor loss (broken blue arrow) and CO2 uptake (broken brown arrow) by diffusion from higher to lower partial pressures”

**Phloem**

The phloem is a tissue that allows long-distance transport (translocation) of photosynthates, or products of photosynthesis. Redistribution of water and other compounds is another one of its functions. Photosynthates must be transported from their source, typically mature leaves, to non-photosynthetic organs and areas that are unable to produce enough photoassimilates to grow, such as roots, young leaves, or fruits. Storage organs, including roots, tubers, rhizomes, can be both sink, when accumulating nutrients, and source, when providing said nutrients to the rest of the plant body during winter or other stress-inducing times (Taiz & Zeiger, ). Seeds do the same, transitioning from source to sink when they germinate and need to demolish the resources that they accumulated as starch to support the new plant’s growth (Rascio *et al.*, 2017).

In Angiosperms, which are the main object of research on the phloem, xylem and phloem form vascular tissues that extend throughout the plant body, with the xylem being usually closer to the centre of the stem (or the root), and the phloem on the outer side (Taiz & Zeiger, ). The two continuously exchange water. %specify primary vs secondary growth?

The phloem is made up of living cells belonging to three different categories: sieve elements, companion cells and parenchymatic cells. In Angiosperms, sieve elements are roughly cylinder-shaped cells that are stacked on top of each other, forming a sieve tube (Rascio *et al.*, 2017). Cross walls separating the cells are perforated by pores, forming sieve plates, to achieve symplastic continuity and a higher hydraulic conductance inside the tube (Peters \& Knoblauch, 2022). As sieve cells do not possess a nucleus, a cytoskeleton and other essential organelles, each sieve cell is associated with one or more companion cells. Plasmodesmata provide a close symplastic relationship between sieve cells and companion cells. The latter have intense metabolic activity and are responsible for the survival of the anucleate sieve cells. They also contribute to the transport of photosynthates from mesophyll cells in leaves, where they are produced, to sieve cells (Rascio *et al.*, 2017).

Phloem sap is rich in sucrose, varying from 0.3 to 0.9 M in concentration. In addition to sucrose, the phloem of certain plant families also contains high concentrations of sugar alcohols like mannitol and carbohydrates like raffinose and stachyose. Other solutes in lower concentrations include amino acids, hormones, proteins and inorganic solutes (Taiz & Zeiger, ).

Long-distance transport in the phloem is usually explained by the pressure-flow hypothesis, formulated by Ernst Münch in the 1920s. The hypothesis states that mass flow of water and solutes in the phloem is driven by an osmotically induced pressure gradient between source and sink organs. The loading of photosynthates into the sieve elements at the source causes a low solute potential, which in turn causes a negative water potential, so that water is drawn into the phloem from neighboring xylem tissue. The opposite phenomenon happens in the sink organs, where photoassimilates are unloaded from the sieve elements and water potential becomes more positive, leading water to leave the phloem as well. This creates a difference in turgor pressure, which is higher at the source, pushing the fluid along the phloem, and lower at the sink (Knoblauch et al, 2016).

The phloem has been called “the least understood plant tissue” (Knoblauch et al, 2016). Despite more than a century of research, some problems remain unsolved. An interesting area of studying concerns carbon allocation and partitioning. Research on the phloem has been conducted through methods like pulse labelling and girdling. Studying carbon allocation – review di Epron, 2012;

Rascio:

Starch: only synthesized in chloroplast. Process that uses ATP 🡪 Pi released and sustains ATP synthesis (photophosphorylation). Starch is low in the morning, accumulates during the day and is then demolished during the night –> result = carbs that are exported in cytosol and used for respiration or converted into sucrose.

Sucrose: synthesized in cytosol. In order to exit the chloroplast, triose phosphates go thorough triose phosphate transporter in inner chloroplast membrane. This is an antiport that lets triose phosphates out and inorganic phosphate in. Inorganic Pi comes from sucrose synthesis in the cytosol, which follows TP’s exit. As sucrose is not phosphorylated, the Pi in TP is released during biosynthesis and is used by the TPT antiport.

Phosphosynthates allocation = regolazione tra più vie alternative per quanto concerne utilizzo della sostanza organica: accumulo, trasporto, metabolismo (respirazione + scheletri carboniosi).

~~Taiz/Zeuger: allocation = regulation of the diversion of fixed carbon into the various metabolic pathways. Partitioning = differential distribution of photosynthates within the plant.   
Sucrose synthesis in the cytoplasm diverts triose phosphate away from starch synthesis and storage. For example, it has been shown that when the demand for sucrose by other parts of a soybean plant is high, less carbon is stored as starch by the source leaves.~~

~~Sink strength (ability of a sink to mobilize photosynthate toward itself) = sink size × sink activity~~

~~Sink size is the total weight of the sink tissue, and sink activity is the rate of uptake of photosynthates per unit weight of sink tissue.~~

~~Cooling a sink tissue~~ **~~inhibits activities that require metabolic energy~~** ~~and results in a decrease in the speed of transport toward the sink.~~

~~If all but one of the source leaves of a soybean plant are shaded for an extended period (e.g., 8 days), many changes occur in the single remaining source leaf. These changes include a decrease in starch concentration and increases in photosynthetic rate, rubisco activity, sucrose concentration, transport from the source, and orthophosphate concentration (Thorne and Koller 1974). These data indicate that, besides the observed short-term changes in the distribution of photosynthate among different sinks, the metabolism of the source adjusts to the altered conditions in long-term experiments.~~

~~Turgor pressure in the sieve elements could be an important means of communication between sources and sinks, acting to coordinate rates of loading and unloading. Chemical messengers are also important in signaling to one organ the status of the other. Such chemical messengers include plant hormones and nutrients, such as potassium and phosphate and even the transport sugars themselves.~~

Carbohydrate levels can influence the expression of encoding photosynthesis component genes, as well as genes involved in sucrose hydrolysis. Many genes have been shown to be responsive to sugar depletion and abundance. In sugar beet, for example, proton–sucrose symporter activity declines in plasma membrane vesicles isolated from source leaves fed exogenous sucrose through the xylem. Decline in symporter mRNA (article found). (Let’s not do that)

A working model includes the following steps: (1) Decreased sink demand leads to high sucrose levels in the vascular tissue; (2) high sucrose levels lead to down-regulation of the symporter in the source; (3) decreased loading results in increased sucrose concentrations in the source (Chiou and Bush 1998). Increased sucrose concentrations in the source can result in a lower photosynthetic rate.

~~Sink strength has been defined as the ability of a sink organ to import photoassimilates, which is co-regulated by sink size (the total biomass of sink tissue) and activity (specific uptake rate of the resource)~~

**Aim of the thesis**

This thesis shows part of the results for an experiment about the effects of sink limitation on (\textit{Betula pendula}) carbon translocation. It was conducted in the framework of Xin Zhuang’s Doctorate work.

Sink activity reduction was achieved by cooling the soil around the roots of 12 saplings, while 8 other trees were left untreated to function as controls. One goal of the experiment was studying how the sink limitation created by soil cooling affects leaf photosynthetic activity, by measuring gas exchange parameters such as CO2 assimilation and photosynthetic efficiency (Zhuang et al, ?). I am also going to report on the results of the water status monitoring (water potential monitoring) during the experiment.

Questions:

* Abstract: “We hypothesize that sugar accumulation causing increasing mesophyll resistance is the bottleneck for photosynthesis and for phloem loading when plants have a restricted carbon sink.” – **cosa vuol dire? –** discorso uguale a quello che fanno in Sink regulation of photosynthesis. OK
* Significance of the xylem/ water potential becoming lower as we limit the sink? Hydraulic status? Stress parameter? When you cool roots, less water is transported up (water capacity is limited) – so we are trying to see if there
* Significance of gsw and E? Is it simply because they indicate that less CO2 is able to get in?
* Do I need to write about why photosynthesis is downregulated?
* Do you think it is worth it to go into depth about research on phloem transport? Girdling/ labelling, phloem translocation, etc. are not the focus of my thesis. The focus is effect on source activity! So maybe it is better to just put stuff about that? Yes, it is.
* “**By calculating soil to leaf hydraulic conductance, we were able to separate the effect of sink limitation on gas leaf exchange from the effect of decreased water uptake capacity of the roots due to the reduced temperature**” -> would be important to mention this in Discussion. OK

the effect of cooling on xylem sap flow velocity with a Sap Flow Meter (removed)

**Materials and methods**

**3.1 General information**

The experiment took place at the Viikki Campus (Department of Forest Sciences, Faculty of Agriculture and Forestry), at the University of Helsinki, in a greenhouse environment, starting from 20th June 2023 and ending on 10th August of the same year. (standard temperature and humidity?). The conditions in the greenhouse were on average 21°C and 60% relative humidity. I was able to witness and work on the experiment during my time as a trainee at the University of Helsinki, under the mentorship of Xin Zhuang and the supervision of Dr. Yann Salmon.

The setup for the experiment involved 20 birch saplings *(Betula pendula*, age? Height and diameter?) which were replanted in (type of soil? Pot dimension?) and placed in the greenhouse a week before the start of the measurements.

The LAUDA thermostat was used to bring temperature down to 4 +/-3 °C, with the aim of studying the effects of the cooled-down roots on the physiology of the trees. The thermostat consists of a refrigeration unit and a tubing system, which was filled with anti-freeze. The silicone tubes were buried in the pots so that they were closer to the roots, cooling them more efficiently. Further isolation was achieved by putting the pots into *bags* (how do you call those?) and wrapping foam around the tubes. (Further info? Separate chapter?)

Measurements were taken on 5 trees at a time. Every group consisted of two control trees and three treated trees that had been cooled down. The first group was measured from 28th June to 13th July; the second group was measured from 13th July to 1st August; the third group was measured from 1st August to 7th August; the fourth group was measured from 7th August to 10th August. Devi spiegare meglio qual è la funzione del raffreddamento, cosa vuoi dimostrare?

The instruments used for the measurements I will discuss in this thesis were the LI-6800 (LI-COR’s fourth generation portable photosynthesis system), a pressure chamber (ask for manual) for water potential, and (instrument for temp and sap flow?). Cambia qui: parti da misurazioni, poi vai agli strumenti.

**3.2 Gas exchange**

(Brief explanation of how it works (manual) + how it was used in experiment) OK

The LI-6800 can measure photosynthetic processes happening at leaf-level without destroying the sample. Several leaf chambers of different dimensions are available; for this experiment, chamber was used.

After the chamber is closed onto a leaf, two parameters, assimilation (*A*) and evapotranspiration (*E*), can be measured with infrared gas analysers. This is based on the difference in and between the air in the leaf chamber, which is affected by the leaf, and air that is not (reference). Other parameters, e. g. stomatal conductance (*gsw*) and intercellular concentration (), are calculated thanks to other measurements taken by LI-6800, including leaf temperature.

Come hai usato gas exchange per analizzare raffreddamento?

**3.3 Water potential**

Cheat sheet for ggplot2 – data visualization with ggplot2

The idea of the data is to show it (what we measured) and then add the statistical analysis.

sci-hub

* Cerca articoli sul resto dell’intro + potenziale idrico

Domande:

* Batch and treatment for SHR, how do I explain? Source: Sokal, R.R. and Rohlf, F.J. (1995) Biometry: The Principles and Practice of Statistics in Biological Research. 3rd Edition, W.H. Freeman and Co., New York.

gam1<-gam/y~x...)

plot(resid(gam1)~predict(gam1))

GAM:

* prova di nuovo gam e quel codice
* <https://r.qcbs.ca/workshop08/pres-en/workshop08-pres-en.html#95>
* pag 44 + pag 47 per gam NO
* altre 2 pagine nei preferiti
* extrapolate from ggplot (kind of what I did? Library(mgcViz)) OK

Validation tools for the GAM model that contains one smoother for depth and a nominal variable ID (= station). The QQ-plot and the histogram are used to assess normality and the residuals versus fitted values homogeneity. The response against fitted values should ideally show a straight line. Pag 59 Zuur (page 76 not pdf).

Page 77-8 (pdf): he is saying that the “right two panels” are now better (even though there is still a pattern!).

Cose da approfondire forse:

* Phloem loading
* Dove stanno/ vengon prodotti starch e sucrose (impo per capire il processo di feedback)

Stomata explanation: page 81 pdf (ch 4)

Cloroplasti in Rascio non sono spiegati perché la pagina manca, ops.

Scatter plot E vs water potential ok

model1<-(E~batch + WP \* Ts)

model2<-E~bact + WP + Ts

AIC(model1,model2) – R square with a cost for every extra variables

Summary(model1)

Anova(model1, model2) – no significant difference? I select model 2 because it doesn’t do a worse job at explaining data and has a lower AIC.

Last advice:

* E ~batch + Water potential \* soil temperature
* ~~Page 345 + 137 Lamberts – soil and temperature and growth~~
* ~~Non-stomatal limitation: mesophyll conductance, stress that can inactivate Rubisco or make photorespiration bigger (even accumulation of sugars can do that). Si vede impatto di non stomatal limitation da A/Ci. Ci diminuisce quando gs diminuisce. Se faccio A/Ci, tolgo quel fattore.~~

Because of capillarity, which aims at restoring the equilibrium, water is pulled against gravity and negative pressure, i. e. tension, is generated. This decrease in hydrostatic potential and water potential is then transmitted to neighboring areas, and eventually to the whole xylem and the roots

Young-LaPlace equation

~~Correggi assimilation in assimilation rate, evapotranspiration rate, stomatal conductance to water vapor~~

~~Put A, A/Ci, E, gsw (ha più senso)~~

~~Descrivi per bene le 4 variabili con licor manual come supporto~~ + ~~Photosynthetic efficiency (non-stm cond?)~~

Cambia tutte quelle parentesi e slash, in math mode $

Riformula parte dal manuale licor + parte del floema

~~Aggiungi stomi~~ e ~~parte sulla conduttanza idraulica~~

~~Paragrafo soil cooling: metti assieme info su come stomatal conductance influenzi fotosintesi + su come soil cooling influenzi water pot e dunque di nuovo st cond e phot (ma non per feedback del sink!)~~

~~Metti figura diagnostics plot subito dopo gamma, perché mostra dp di gamma e non di gam (oppure metti dp di gam e non di gamma, perché c’è il grafico della non normalità, ecc). cerca definizione residuals~~

~~Packages R: nomi e reference~~

Results: ~~integra le parti precedenti (rileggendo aim of thesis~~); alcuni batch da soli NON sono significativi?? Percentuali di diminuzione a seconda di calo temperatura?

Arricchisci captions (Sicura che il carattere e gli spazi siano giusti?)

The discussion section, which follows the results section, will include an explanation of the results. In this section, you should connect your results to previous research studies, make explicit connections back to your research question(s) and include an explanation about how the results might be generalized.

Conclusioni: parla di a/ci come non stom cond info. Le prime due parti erano as expected. La terza no: further research is needed on water pot + on sugar per le prime due parti. Eventualmente metti relazione tra water pot e soil t.

Spiega cose nei risultati: confermata ipotesi per parte 1 e 2; risultato inaspettato per terza parte. Metti prima grafico con tutto assieme, poi fai distinzione. Tutto assieme (unica riga) con divisione cooled/ control?

Allora, soil T NON ha avuto effetto su water p 🡪 w pot probabilmente è stato influenzato da qualcos’altro che non sappiamo e diminuisce mentre valori salgono (inaspettato). Aggiungi quali batches non sono significativi individualmente?

Sistema codice da metter in fondo!

Ricontrolla correzioni Zaffagnini

Domande:

* Titolo
* Come fare dev standard (prima parte analisi)
* Definizioni variabili? Photosynthtic efficiency ok
* Hydraulic conductance e soil cooling ok

Definizione di hydraulic conductance: parte sul potenziale idrico (+immagini)

* Stomata: collegamento con fotosintesi e water potential
* Root cooling: provoca diminuzione di fotosintesi (artemisia), ma anche diminuzione di hydraulic conductance (e water potential)

1. Bekker, A. et al. Dating the rise of atmospheric oxygen. Nature 427, 117–120 (2004).

2. Trebst, A. V., Tsujimoto, H. Y. & Arnon, D. I. Separation of Light and Dark Phases in the Photosynthesis of Isolated Chloroplasts. Nature 182, 351–355 (1958). ok

3. Duysens, L. N. M., Amesz, J. & Kamp, B. M. Two Photochemical Systems in Photosynthesis. Nature 190, 510–511 (1961). ok

4. Hill, R. & Bendall, F. Function of the Two Cytochrome Components in Chloroplasts: A Working Hypothesis. Nature 186, 136–137 (1960). used

5. Mitchell, P. Coupling of Phosphorylation to Electron and Hydrogen Transfer by a Chemi-Osmotic type of Mechanism. Nature 191, 144–148 (1961). ok

6. Johnson, M. P. Photosynthesis. Essays Biochem. 60, 255–273 (2016). used

7. Bassham, J. A. et al. The Path of Carbon in Photosynthesis. XXI. The Cyclic Regeneration of Carbon Dioxide Acceptor. 45. Ok

8. Benson, A. & Calvin, M. The Dark Reductions of Photosynthesis. Science 105, 648–649 (1947). I don’t have this because Science is a gatekeeper

9. Ellis, R. J. The most abundant protein in the world. Trends Biochem. Sci. 4, 241–244 (1979). Ok

10. Raven, J. A. Rubisco: still the most abundant protein of Earth? New Phytol. 198, 1–3 (2013). Ok

Stomata are pores in the epidermis of leaves. Each pore is surrounded by two guard cells that can sense temperature, humidity, intracellular $CO\_2$ concentration and other factors and respond by changing their turgor pressure, which leads to the opening or closing of the pores (Taiz \& Zeiger, 2002). Plants lose water ($H\_2O$ evapotranspiration) and take up $CO\_2$ through stomatal apertures, therefore regulating the size of the stomatal openings is essential for them.

While the relative water vapor concentration in the mesophyll is greater than its concentration in the atmosphere, due to xylem activity, the opposite is true for $CO\_2$ concentration, which is lower in the leaf (ref licor manual). These concentration gradients control the water and $CO\_2$ movements. and are maintained by stomatal resistance to gas diffusion

Micrograph of kidney-shaped guard cells (CG) surrounding the stomatal pore. Image taken from Rascio, 2017.

Stomatal conductance; numerical measure of the rate of diffusion of water vapor, carbon dioxide or other gases through the stomatal pore.

Hydraulic conductance in the **root** is flow rate / water pot 🡪 root hydraulic conductance **decreases** with soil cooling (source?) 🡪 soil to leaf conductance decreases 🡪 lower w pot values 🡪 lower gas exchange values.

The water has more trouble moving to the leaf. Temperature decreasing makes roots less active, it is more difficult to take up water. Decrease in water supply the roots are

Gross assimilation = photosynthesis

Net assimilation = photosynthesis – respiration

~~Reason we do that: that removes the effect of stomatal conductance. CO2 inside the leaf accounts for the stomatal conductance part. Non- stomatal limitation.~~

~~Everything lost by gas exchange is replaced by sap flow from roots.~~

È tutto collegato: la traspirazione è ciò che viene preso dal suolo. All the water that comes in goes out.

Da Matta et al., 2007 reached a different conclusion regarding their experiment on coffee.

\begin{itemize}

\item complete defruiting and maintenance of the full leaf area

\item half crop load and full leaf area

\item full crop load and full leaf area

\item full crop load and half leaf area.

\end{itemize}

The first treatment resulted in the lowest net photosynthetic rate (\textit{A}) and stomatal conductance (\textit{g}), the third treatment had the highest \textit{A} and \textit{g}, while the other two treatments had intermediate results. However, here a different conclusion is proposed: the decreased \textit{A} in the first treatment is thought to be caused not by direct sink feedback, but by the lower stomatal conductance, as (\textit{A}) and (\textit{g}) were correlated. The reason for the lower \textit{g} remains unknown.

Other than fruits, roots can also be studied as sink organs down-regulating photosynthesis, as this thesis will investigate. As stated in section \ref{sec:source}, cooling down the roots of a tree inhibits its sink activity. This technique is not as disruptive as girdling is: since no tissues are damaged, wound responses do not get triggered, and there is a lower risk of changes in secondary metabolism affecting the experiment.

\smallskip

Soil cooling has been known to limit photosynthesis in several different species like \textit{Artemisia tridentata} (BassiriRad et al., 1993), pine (Day et al., 1991; Lintunen et al., 2020), aspen and spruce (Cai et al., 2001. The inhibition is thought to be caused by both stomatal and non-stomatal limitation. The latter includes mesophyll and chloroplast resistance to $CO\_2$ and metabolic restrictions such as RuBiSCo inactivation (Signarbieux et al, 2010; Salmon et al., 2020).

\smallskip

Day et al., 1991 observed that stomatal limitation may have had a major role in the decrease of net photosynthetic rate as temperature dropped from 24°C to 7°C. This is suggested by stomatal resistance and intercellular $CO\_2$ concentration declining as well. At temperatures below 7°C, intercellular $CO\_2$ concentration increased, but the photosynthetic rate kept decreasing, suggesting non-stomatal limitation. % does this make sense?

\smallskip

Root cooling for \textit{Artemisia tridentata} also resulted in a limitation of photosynthesis. The A (photosynthetic rate) versus $C\_i$ (intercellular $CO\_2$ concentration) curve did not change with temperature, which suggests stomatal limitation (BassiriRad et al., 1993).

\smallskip

There can be multiple causes for stomatal and non-stomatal limitation. While they might be a result of direct sink feedback and sugar accumulation - or simply a consequence of a lower photosynthetic activity, in the case of stomatal limitation -, they might also be connected to a lower hydraulic conductance. When soil temperature goes down, hydraulic conductivity has been shown to also decline. Because the roots take up water less efficiently, the water potential of the entire plant is negatively affected, and stomatal conductance ends up declining as well (Day et al., 1991; Lintunen et al., 2020).

\subsubsection{Isotope pulse-labeling}

For our experiment, the labeled ${}^{13} CO\_2$ was pumped into plastic bags that had been wrapped around the birches' canopy. Labeled carbon was then traced with Picarro's G2201-i Isotopic Analyzer, as it was transported from the source (leaves) to the sink organs, via the phloem. Chambers were sealed at two different locations along the stem and connected to the analyzer, so that the carbon could be traced when it respired. The difference in time lags between the appearance of labeled C at the first and second location can be used to establish phloem sap velocity \citep{Epron\_D.\_et\_al.\_2019}. However, this data is still being processed by Xin Zhuang, as it requires complex analysis, therefore it will not be discussed any further in this thesis.

The experiment also aimed at studying the effect of soil cooling on carbon translocation and not just photosynthetic activity, which is why we used ${}^{13} C$ pulse-labeling to the canopy with the purpose of determining changes in phloem transport velocity between the control trees and the cooled trees.

The difference in canopy source activity between control saplings and treated saplings was estimated through several variables. For leaf photosynthetic activity, four leaf gas exchange variables were measured: $CO\_2$ net assimilation rate (\textit{A}), photosynthetic efficiency (\textit{$A/C\_i$}), $H\_2O$ evapotranspiration rate (\textit{E}) and stomatal conductance to water vapor (\textit{gsw}). $CO\_2$ net assimilation is defined as a measure of the difference between photosynthesis and respiration, while evapotranspiration measures the rate of water loss (ref licor manual). Both variables are connected to stomatal conductance, as $CO\_2$ and $H\_2O$ diffuse through the stomata. Photosynthetic efficiency, which is a normalization of \textit{A} by $C\_i$ (intercellular $CO\_2$), was calculated and used as a variable, in order to remove the effect of stomatal conductance and to have some data about non-stomatal limitation as well. % probably unclear

The four variables provide different information about photosynthesic activity, with \textit{A} and \textit{$A/C\_i$} measuring the rate of photosynthesis directly, and \textit{E} and \textit{gsw} offering more general information about the degree of opening of the stomata.

For tree hydraulic status, we monitored the water potential. The instruments used during the experiment were the LI-6800 (LI-COR’s fourth generation portable photosynthesis system), a pressure chamber for water potential, and

%(instrument for soil temperature, name?)

The experiment also aimed at studying the effect of soil cooling on carbon translocation and not just photosynthetic activity, which is why we used ${}^{13} C$ pulse-labeling to the canopy with the purpose of determining changes in phloem transport velocity between the control trees and the cooled trees.

\smallskip

Table: adding units, fare notazione scientifica, captison sta sopra non sotto

\begin{tabular}{| l | l | l | l | l | l | l |}

\hline

TIME & TREE ID & REPLICATE & \textit{E} & \textit{A} & \textit{Ci} & \textit{gsw} \\

\hline

10:41:36 & BC1 & 1 & 0,001766 & 11,2275 & 244,925 & 0,137237 \\

\hline

10:43:31 & BC1 & 1 & 0,001716 & 11,31848 & 241,3424 & 0,134534 \\

\hline

10:44:43 & BC1 & 1 & 0,001702 & 11,3601 & 239,3733 & 0,133086 \\

\hline

11:00:11 & BC1 & 2 & 0,001906 & 14,15517 & 217,8557 & 0,146854 \\

\hline

11:01:08 & BC1 & 2 & 0,001867 & 14,26866 & 214,0073 & 0,144471 \\

\hline

11:02:04 & BC1 & 2 & 0,001887 & 14,28922 & 215,357 & 0,146011 \\

\hline

11:15:54 & BC1 & 3 & 0,003471 & 12,66993 & 301,7897 & 0,285015 \\

\hline

11:17:30 & BC1 & 3 & 0,003373 & 12,62602 & 301,5399 & 0,282158 \\

\hline

11:18:47 & BC1 & 3 & 0,00331 & 12,59355 & 300,9615 & 0,27851 \\

\hline

11:55:53 & BC2 & 1 & 0,003087 & 14,68526 & 276,676 & 0,250679 \\

\hline

11:56:44 & BC2 & 1 & 0,00312 & 14,71186 & 276,8577 & 0,251933 \\

\hline

11:57:26 & BC2 & 1 & 0,003126 & 14,64707 & 277,4459 & 0,252505 \\

\hline

12:12:56 & BC2 & 2 & 0,002637 & 11,30982 & 290,1498 & 0,212438 \\

\hline

\end{tabular}

\label{table:controls}

\end{table}

\begin{table}

\centering

\begin{tabular}{| l | l | l | l | l | l | l |}

\hline

TIME & TREE ID & REPLICATE & \textit{A} & \textit{E} & \textit{Ci} & \textit{gsw} \\

\hline

12:13:58 & BC2 & 2 & 0,002633 & 11,29484 & 290,7186 & 0,213459 \\

\hline

12:15:17 & BC2 & 2 & 0,0026 & 11,32306 & 289,9024 & 0,211932 \\

\hline

12:31:16 & BC2 & 3 & 0,002889 & 13,70625 & 276,4829 & 0,230113 \\

\hline

12:31:56 & BC2 & 3 & 0,002941 & 13,75695 & 278,1119 & 0,235174 \\

\hline

12:32:39 & BC2 & 3 & 0,002905 & 13,78629 & 277,2324 & 0,233377 \\

\hline

\end{tabular}

\end{table}

Because the distribution of the data as well as of the residuals of the linear regression were not normal, a classical linear regression was not suitable.

QQ-plot or histogram of the residuals for normality