

Metabolic safety margin as an indicator of transient state of the energetic metabolism in trees

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Abstract

Non-structural carbon (NSC) reserves, especially starch, are vital for trees to survive and recover from stress. NSC dynamics leave a footprint in the age of NSC stored and allocated to respiration and growth. We propose a new measurement, the *Metabolic Safety Margin* (MSM), to evaluate the metabolic state of trees and NSC turnover times by analyzing the relationship between NSC mean age and transit time.

NSC mean age is the time from carbon fixation to measurement, while NSC transit time is from carbon fixation to allocation for metabolic needs like respiration and growth. These quantities are modeled using compartmental models and a novel mathematical framework, and estimated by measuring ^{14}C in stored starch or soluble carbon and respired CO_2 .

We modeled the Metabolic Safety Margin for five trees with varying NSC turnover times by adjusting NSC allocation to slow cycling storage compartments. Our models indicate that trees prioritizing storage are further from the 1:1 line and take longer to reach it under starvation. They also show a longer and slower transient response of the MSM to reduced carbon income.

We estimated the age of soluble carbon and respired CO_2 in the stem wood, branches and roots of trees in the amazon with contrasting storage strategies and trees in the the Swiss Alps using ^{14}C . Results suggest that MSM in species with high priority to storage is further from the 1:1 line. Additionally, MSM in roots and stem wood may provide more long term metabolic information than MSM in branches, probably because their slower NSC turnover times.

The Metabolic Safety Margin helps evaluate the metabolic state of mature trees and their resilience to environmental stress, improving estimates of tree vulnerabilities to climate change.

1 Introduction

Trees are long-lived organisms that face and survive multiple and frequent stressors and disturbances along their life-span. Global change is increasing the frequency and severity of stress and disturbances by increasing air temperature, vapor pressure deficit (VPD), and shifting precipitation regimes, affecting trees all over the world. The combination of these factors is expected to cause changes in forest demographics with severe consequences for the global carbon cycle and for the climatic crises we now face. Understanding the

vulnerability of trees and forests to these new set of conditions and their survival and recover is paramount for us to predict changes in forest composition and functionality facing new global environmental conditions.

Tree’s investment into carbon reserves help them to survive and recover from multiple disturbances or stressful changes in environmental conditions. Carbon reserves in the form of nonstructural carbon (NSC) are mainly soluble sugars, starch and lipids. These reserves can be short-term -cycled very fast with a high turnover- or long-term -cycled slower with lower turnover- depending on the organ they are stored and the tree species. Tree species differ in their carbon allocation strategies and some may prioritize allocation to NSC long-term storage over other allocation alternatives such as growth. Thus, trees storing long-term NSC may have very old reserves that, if accessible, can buffer metabolism at multiannual or multidecadal time scales, playing a fundamental role in long term tree survival and recovery.

Under a positive carbon balance, trees’ metabolism is sustained by recent NSC. However, when trees are in a carbon deficit, they access their reserves to sustain metabolism. In these conditions, the carbon allocated to metabolism starts reflecting the contribution of old reserves, becoming progressively older. When trees recover or adapt NSC reserves replenish with new NSC, and allocation to metabolism comes back to rely on younger NSC. Thus, the interplay between multiple disturbances along trees’ life span and trees’ carbon metabolism would leave footprints in the composition of ages of the NSC reserves and the NSC being allocated to metabolism.

We lack a mechanistic understanding of how stresses and disturbances impact the use of carbon reserves and the trees carbon fluxes that would determine the age composition of the carbon reserves and the age of the carbon allocated to metabolism. Despite its importance, there are few studies that characterize the age and turnover time of NSC in mature trees under stress or when facing multiple disturbances. This is because the lack of techniques that allow us to estimate NSC turnover times in mature trees, and because keeping track of changes in trees’ carbon fluxes over time is expensive and time consuming. Evaluating the footprints that multiple disturbances may leave on the NSC age composition would be a feasible alternative to investigate how stress impact the use and recovery of NSC reserves. This would help us to estimate how much a tree has been metabolically affected by past disturbances and how vulnerable they may be to the upcoming ones.

Here we want to present a new metric to evaluate the status of carbon metabolism of trees and their vulnerability to future stressful condition. The metabolic safety margin, as we call it, is the relationship between the age of the NSC allocated to metabolism and the age of the store NSC in trees (Fig. 1). This metric serves as a proxy to understand the interplay between trees carbon balance and changes in environmental conditions. We hypothesize that this relationship integrates the metabolic history of an adult tree over the recent years and would indicate how much a tree has been affected by past multiple perturbations and how vulnerable it will be to future extreme events or changes in growing conditions.

2 Metabolic safety margin: a new metric to evaluate the metabolic status of mature trees

Carbon reserves (NSC) are thought to be rapidly cycled in trees. Nevertheless, turnover times of NSC can vary from organ to organ and from species to species. For instance, they are cycled at a daily basis in leaves, but they may be multiannual in the long term reserve pools such as the stemwood. The time that they can be stored in the stemwood highly depends on the species and the wood anatomy. NSC turnover time also depends

on the pool size and the carbon fluxes towards metabolic needs such as respiration and growth. Besides, this NSC turnover time would vary as trees regulate their carbon fluxes as function of environmental conditions. The strategy for the storage and use of long term reserves affects trees carbon fluxes and metabolism, which reflects on the capacity of trees to survive certain disturbances or stressful conditions and recover.

Estimates of the age of the NSC stored in trees could serve as a proxy for the NSC turnover time, if trees are in steady state. Age of the NSC can be defined as the time between the carbon fixation by photosynthesis until the time of observation. Large carbon allocation to NSC reserves and low carbon allocation to growth, respiration and reproductions would result in low turnover times. The lower the NSC turnover time the older NSC would become in the reserve pools. Nevertheless, NSC turnover time have high variability. There are, in nature, trees with very fast NSC turnover which results in very young stored NSC and trees with reserves that have been stored for decades. NSC turnover time may also vary along trees life span or may change in response to environmental stressors, although changes in NSC turnover time may imply changes in the carbon allocation strategy. Some of these stored reserves, old or young, remain available over time to support metabolism and growth when trees face stressful conditions or have to recover from disturbances, playing a fundamental role in trees resilience to environmental changes.

As trees are not usually in steady state, and NSC pool size and carbon fluxes changes with environmental conditions, the observed age of the NSC alone may not be a good proxy of the NSC turnover time. Furthermore, NSC ages are not homogeneously distributed in trees or tree organs. The distribution of ages may depend on the mixing rates between old stored NSC and newly assimilated carbon, which may be determined by trees life history traits. Fluctuations of the carbon balance and the carbon mixing rates influence the age composition of the NSC reserves and create transient responses on the NSC ages that are not the reflection of changes in the NSC turnover time.

These differences in the NSC age distributions and fluctuations in the carbon balance in trees make the age of the allocated carbon to metabolism differ from the age of the NSC stored in the reserve pools. NSC transit time is the age of the carbon since it enters the tree until it is allocated to other carbon sinks rather than NSC (e.g. respiration, growth, reproduction or exudates). Then, here we can regard the age of the NSC allocated to metabolism and the NSC transit time. Thus, for mature trees that face multiple disturbances we would expect that the average mean age of the NSC in the a tree is different to the average NSC mean transit time. The relationship between these two metrics and its changes over time can give us insights about the metabolic state of trees and their response to multiple disturbances.

Trees under a positive carbon balance would usually allocate a larger proportion of young carbon to metabolism. Usually carbon allocated to respiration and growth in healthy trees range from 1 to 5 years of age, on average. When trees are under a negative carbon balance, they access carbon reserves to sustain their metabolism and survive. As disturbances or stress progress, the contribution of old reserves to metabolic functions progressively increases. This contribution of old reserves to trees metabolism would be reflected in the NSC transit time. As stress progress in trees, NSC transit time becomes progressively older as much as the age composition of the available reserves allows it. This progression help us understanding how fast trees can access their reserves and for how long carbon reserves in trees remain available, helping us to better understand transient responses of NSC ages in trees in response to environmental changes.

Then, the relationship between the NSC mean transit time and the NSC mean age at a given time may help us to estimate how much of the long term reserves are contributing to trees metabolism, relative to the NSC turnover times of individual trees. For instances, when trees cycle NSC very fast and do not store NSC for long periods of time, this ratio would always be very close to 1, meaning that due to the fast NSC turnover time

trees reserves would not last very long under extreme stressful conditions that result in a negative carbon balance.

When trees have slower NSC turnover time, they would have older stored NSC. In healthy conditions, these trees may have very young transit times and old reserves, making the relationship between the NSC mean transit time and NSC mean age smaller than one. Nevertheless, under stressful conditions trees may start accessing these reserves and the transit time would become older leading this relationship closer to 1. This transient response show us how much trees are relying on the old storage to maintain their metabolic functions. When the relationship between the transit time and the age of the stored NSC gets closer to 1, it may indicate an exhaustion of carbon reserves. This can happen because a transient change in the composition of NSC age or because a change in the NSC transit time, or both. These changes may indicate a more vulnerable state of trees to upcoming disturbances.

We propose here using this relationship between the NSC mean transit time and NSC mean age and its relative distance to the 1:1 line as a metric to evaluate the trees vulnerability to stress and disturbances, and their capacity to recover (Fig. 1). We named it “metabolic safety margin” and would serve as a proxy of the transient change in NSC age composition or in NSC turnover time that trees may experience in a given moment, and would help us evaluate the metabolic health of adult trees.

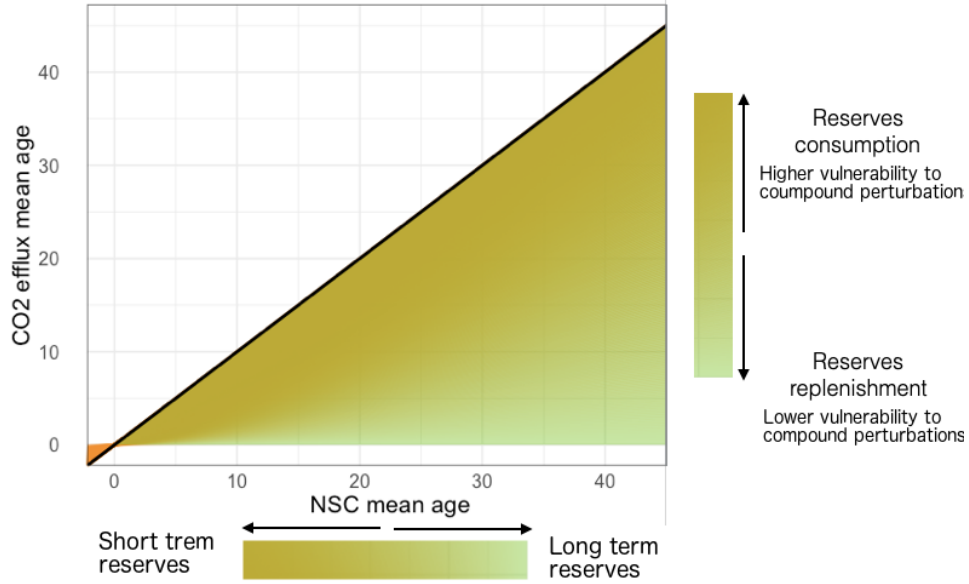


Figure 1: Theoretical relationship between the NSC mean age and the NSC transit time. Darker colors show a closer proximity to the 1:1 line which indicates a faster NSC turnover time

3 Estimates of the metabolic safety margin

Estimating NSC mean age and NSC mean transit time for entire mature trees is challenging, but we can consider good proxies to rely on for estimating and measuring these two quantities. For insntace, ^{14}C measurements in the soluble carbon, starch, respired CO_2 ,

and new wood formed after cathastrophic events have been used to estimate NSC ages and allocation of carbon to metabolism during stress. Alternative, these quantities have also been modelled. Models allow us to estimate the NSC mean ages and transit time in specific organs and in the entire tree. They also allow us to estimate the distribution of NSC ages and transit time and their variability between tree species and tree organs. In the following sections we will illustrate how both thecniques help us estimating and understanding the metabolic safety margins.

3.1 Modelling approach

Metzler et al 2018 have developed an inovative mathematical framework to estimate mean ages and mean transit time for compartmental systems. This framework have been recently used in several studies to estimate differences in age composition of NSC in trees with different storage strategies or growing at different environmental conditions. One way to explain carbon allocation is dividing NSC in several compartnets in trees. Carbon gets into the system as sugars fixed from photosynthes that are distributed to several compartmentes such as leaves, stem and roots. Then, in each organ it may be allocated to NSC reserves, respiration, biomass, reproduction, defense compounds or exudates. We can use this conceptual compartmentalization of NSC allocation to estiamte the NSC mean ages and transit times for each defined compartment and for the whole tree using the matematical framework proposed by Metzler et al 2018.

As an example, to estimate the mean age and mean transit time of NSC of trees with different allocation strategies and its changes following stressful conditions or disturbances, we used the NSC compartmental model adapted from Herrera-Ramirez et al 2020 and Klein and Hoch 2014 (Fig. 2). This model considers leaves, stems and roots as organ comprtments and in each of these organs it considers two NSC compartments: stored NSC (slow cycled) and active NSC (fast cycled) (Table 1). For simplicity, only allocation to growth and respiration was considered as the ouflux of carbon from each compartment/organ (Fig. 2. With these models we wanted to explore the differences in the metabolic safety margin of trees with different storage strategies and different NSC turnover times and describe their rspnse to stressful conditions such as starvation or photosynthetic reduction.

Table 1: Compartments description for the model structure proposed in Fig. 2

Abbreviation	Name
E	Transient Carbon Pool
FANSC	Foliage Active Non Structural Carbon
FSNSC	Foliage Stored Non Structural Carbon
SANSC	Stem Active Non Structural Carbon
SSNSC	Stem stored Non Structural Carbon
RANSC	Fine Root Active Non Structural Carbon
RSNSC	Fine Root Stored Non Structural Carbon

We parametrized these models to simulate trees with different storage strategies Table. 2. Parameters in these models represent the annual fraction of carbon leaving each pool, which can be estimated as the flux of carbon out of the pool divided by the size of the pool. We simulated a tree with a very high NSC turnover time (1 year) where all parameters have a value of 1. Then we simulated trees with slower NSC turnover time by changing the parameters of allocation to storage to respecto of the model with NSC turnover time of 1 (Table. 2). Thus we generated four more modeles with varying NSC turnover time as follow: 1) allocation to stem storage; we increased the allocation to storage in the stem

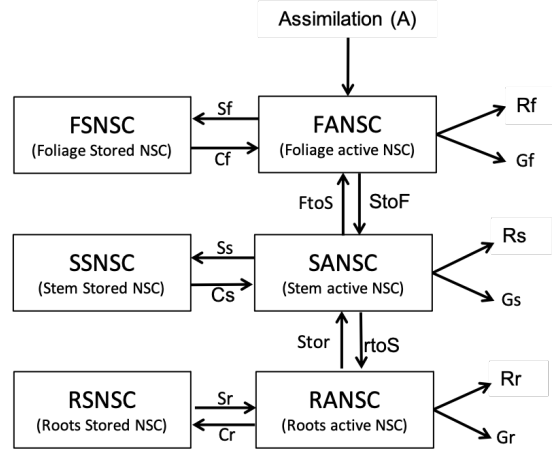


Figure 2: NSC allocation compartmental model. This model considers three organs foliage, stem and roots. In each organ there are two NSC compartments an Active fast cycling compartment and a storage slow cycling compartment. Compartments are indicated by boxes, while fluxes out and into compartments are indicated by arrows. Abreviation names are explained in Table 1 and Table 2

wood by a factor of 60; 2) high allocation to stem storage; we increased the allocation to storage in the stem wood by a factor of 120, 3) high allocation to root storage; we increased allocation to storage in the stem wood by a factor of 60 and we the allocation to storage in the roots by a factor of 120; and 4) low allocation to stem storage; we increased the allocation to storage in the stem wood by a factor of 30. These modifications allow us to evaluate the mean age and mean transit time of trees with different storage strategies and different NSC turnover times at steady state.

Table 2: Annual rates of carbon cycling ($year^{-1}$) and other parameters for each species from the models in Figs 2. Pool name abbreviations are defined in the table 1

Abbreviation	Name	1 year turnover time	stemstorage	High stem-storage	High root storage	low stem-storage
A	Assimilation	230000	230000	230000	230000	230000
Rf	Respiration foliage	1	1	1	1	1
Rs	Respiration stem	1	1	1	1	1
Rr	Respiration roots	1	1	1	1	1
Gf	Growth foliage	1	1	1	1	1
Gs	Growth stem	1	1	1	1	1
Gr	Growth roots	1	1	1	1	1
Sf	Allocation to storage in foliage (FSNSC)	1	1	1	1	1
Ss	Allocation to storage in stem (SSNSC)	1	60	120	60	30
Sr	Allocation to storage in roots (RSNSC)	1	1	1	120	1
Cf	Remobilization from storage in foliage (FSNSC)	1	1	1	1	1
Cs	Remobilization from storage in stem (SSNSC)	1	1	1	1	1
Cr	Remobilization from storage in roots (RSNSC)	1	1	1	1	1
FtoS	Allocation from foliage to stem	1	1	1	1	1
StoF	Allocation from stem to foliage	1	1	1	1	1
Stor	Allocation from stem to roots	1	1	1	1	1
rtoS	Allocation from roots to stem	1	1	1	1	1

We estimated NSC mean age and NSC mean transit time at steady state, for each of the 6 modeled tree types (Fig. ??). These points represent the metabolic safety margin of trees with contrasting storage strategies at steady state. Here we could observe that lower NSC turnover times (represented by higher allocation of carbon to storage in wood and roots, red and blue points in Fig. ??) resulted in older NSC mean age and younger NSC mean transit time relative to storage than trees with faster turnover time (brown and black points, Fig. 3). Thus, the closer the relationship between the NSC mean age and the NSC mean transit time is to the 1:1 line the faster the turnover time of NSC.

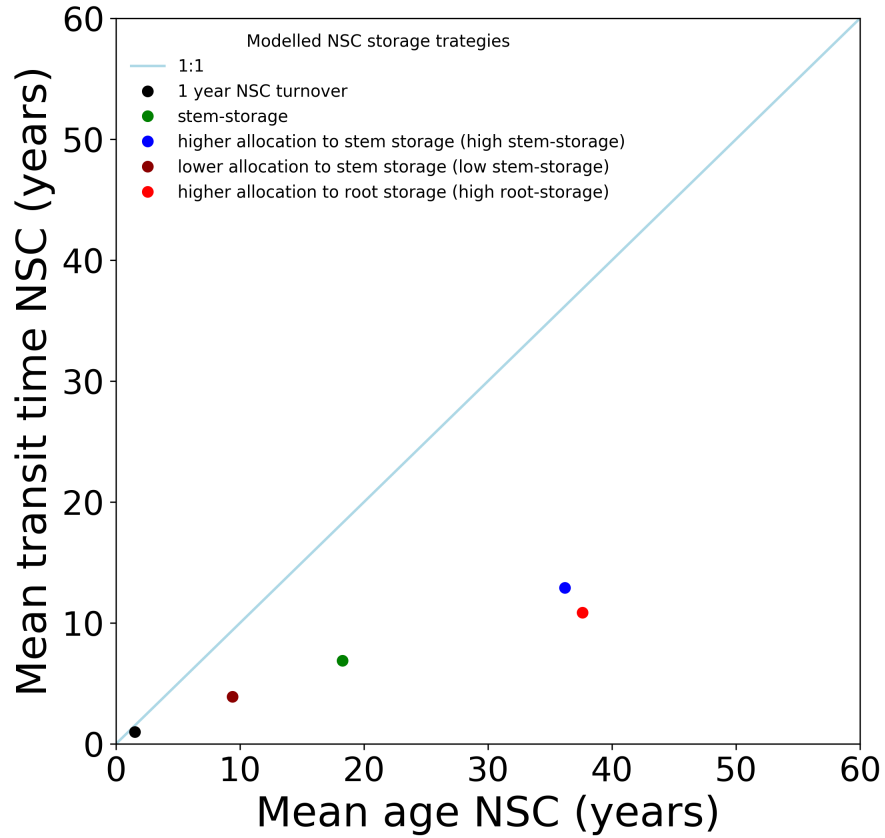


Figure 3: Metabolic safety margin simulated for 5 trees with different NSC turnover times using the model structure presented in Fig. 2 and the parameters described in Table 2 to generate each of the points shown in the model.

We estimated the trajectories of the metabolic safety margin when trees with different storage strategies face starvation. We observed that all models show a trajectory towards the 1:1 line while the system was getting depleted of carbon (Fig. 2). These trajectories approach differentially to the 1:1 line depending on the NSC allocation strategy. For instance, high priority to storage resulted in slower approach to the 1:1 line, with a longer pathway for the larger allocation of NSC to roots (red line) than the larger allocation to stem storage (blue line) (Fig. 2). Meanwhile, lower priority to NSC storage and faster NSC turnover resulted in a fast approach to the 1:1 line (green, brown and black line).

Fig. 2). This progressive approach of each line to the 1:1 line reflects the incremental contribution of the stored reserves to respiration and growth.

These models are linear and not time dependent, therefore NSC turnover time do not change during the simulated starvation, and the estimated changes in the NSC mean age and transit time are a transient response of the age composition of the stored NSC to the simulation of starvation. These changes in the metabolic safety margin give us insights about the ability of trees with different storage strategies to use the stored carbon when facing starving conditions and the amount of time they would take to exhaust NSC reserves, assuming no changes in the NSC turnover times. Changes in the NSC turnover time can happen when trees respond to environmental conditions, reflecting a change in the tree's carbon allocation strategy. Changes in the NSC turnover time would result in changes in the metabolic safety margin line accelerating or slowing down its progression towards the 1:1 line. Thus, progressions towards the 1:1 line in our simulation may appear as an increase in the turnover time as trees exhaust their reserves. Therefore, we can consider these changes as an apparent change in the NSC turnover time, which will inform us about the use or replenishment of NSC reserves over time and the metabolic state of trees in a given point in time.

We also modeled the transient changes in the metabolic safety margin when trees experience a limitation in the carbon supply. In these simulations, income was kept as in Table 2 for the first 10 years and then it was reduced to 2300 gC/year for the next 40 years. Here we compare a fast cycling tree (black line) with a slower cycling tree (green line) (Fig. 5). A reduction of the carbon income produce a transient response in the metabolic safety margin for both trees (Fig. 5). Metabolic safety margin approached the 1:1 line as trees get depleted of carbon, and then they come back to steady state while all the carbon gets replaced by new carbon under the new conditions (Fig. 5). In these new conditions the size of storage pools and carbon fluxes adapt to the new income keeping NSC turnover times constant. Depending on the NSC turnover some trees will take longer than others to come back to the steady state metabolic safety margin, the transient changes in the metabolic safety margin are faster in trees with faster turnover times (black line) than in trees with slower NSC turnover times (green line).

If a tree experiences increases in the NSC turnover times due to changes in environmental conditions, we would expect a shift in transient pathway of the metabolic safety margin from a slower (green line) to a faster (black line) response (Fig. 5). Then, when trees come back to steady state they would be closer to the 1:1 line as NSC turnover time increased. These simulations help us understand the trajectories of the transient response of the metabolic safety margin to stress and perturbations, and help us to evaluate how changes in NSC turnover times would affect trees responses to stress. This provides us a tool to evaluate trees' health and trees' potential ability to survive and recovery under stress and perturbations.

3.2 ^{14}C approach

^{14}C have been widely used to estimate the age of NSC (soluble carbon and starch), the formation of new structural tissues (leaves, wood, roots) and the respired CO_2 in plants. Using the bomb spike in the ^{14}C concentrations in the atmosphere, caused by the bomb testing in the 1950's and 1960's, ^{14}C measurements can be calibrated to calendar dates with only 1 year of uncertainty. Considering only NSC in plants, we can assume that the age of the NSC could be estimated by the ^{14}C signal of the stored NSC (soluble sugars, starch and lipids), while the NSC transit time could be estimated with the ^{14}C signal of the carbon allocated to build new biomass (growth) and allocated to respiration (CO_2).

Estimates of the NSC ages based on ^{14}C gives an average value of the distribution of the NSC age in the analyzed sample. This may not represent well NSC age distribution

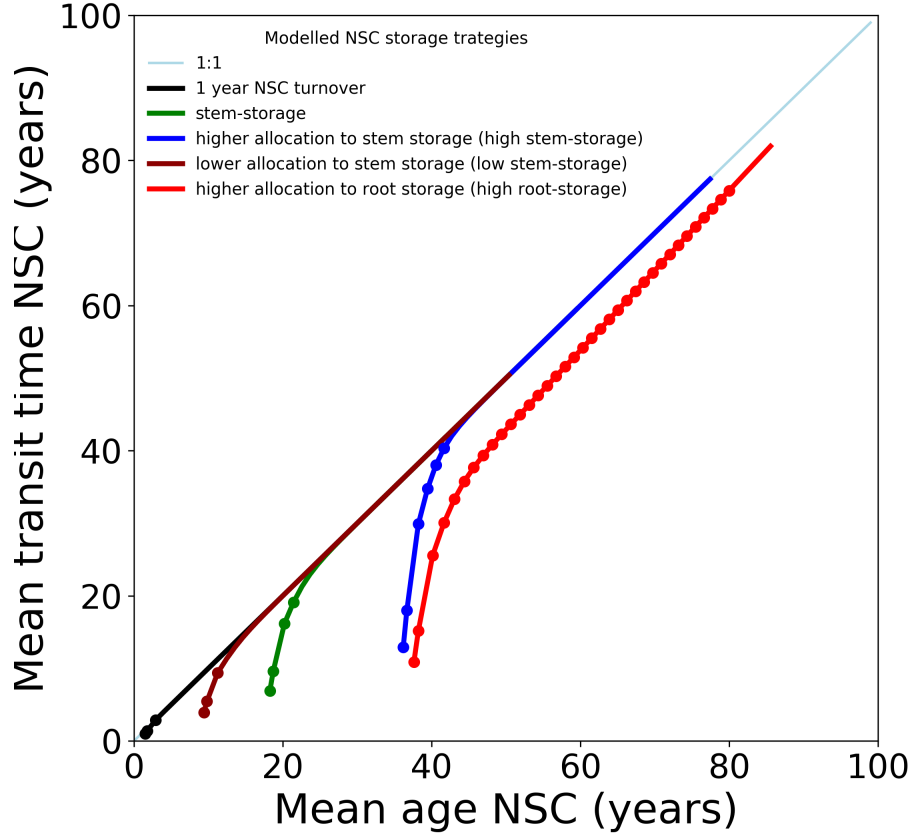


Figure 4: Simulation of the pathway that the metabolic safety margin would follow when trees face starvation for each of the simulated trees specified in the Table2. Each point in each trend line represents one time step of one year. The amount of point each line has before reaching the 1:1 line shows the amount of years each of those trees would take to exhaust their NSC reserves

as they may be skewed distributions towards younger values of NSC ages, and makes estimation of the NSC ages for the entire tree difficult. Nevertheless, to date, ^{14}C measurements are our best approach to characterize mean NSC ages and transit times in tree organs and compounds, and observe their changes when trees face stress or perturbations. It is important to identify what are the key organs and compartments where estimates of the metabolic safety margin would give us insights about the metabolic state of the whole tree, helping us to estimate the metabolic vulnerability of trees to upcoming stressful conditions.

3.2.1 What organs should we measure to estimate trees' metabolic safety margins?

^{14}C ages are not evenly distributed in trees, different tree organs have different age composition depending on trees species and organ dynamics. For instance, leaves will

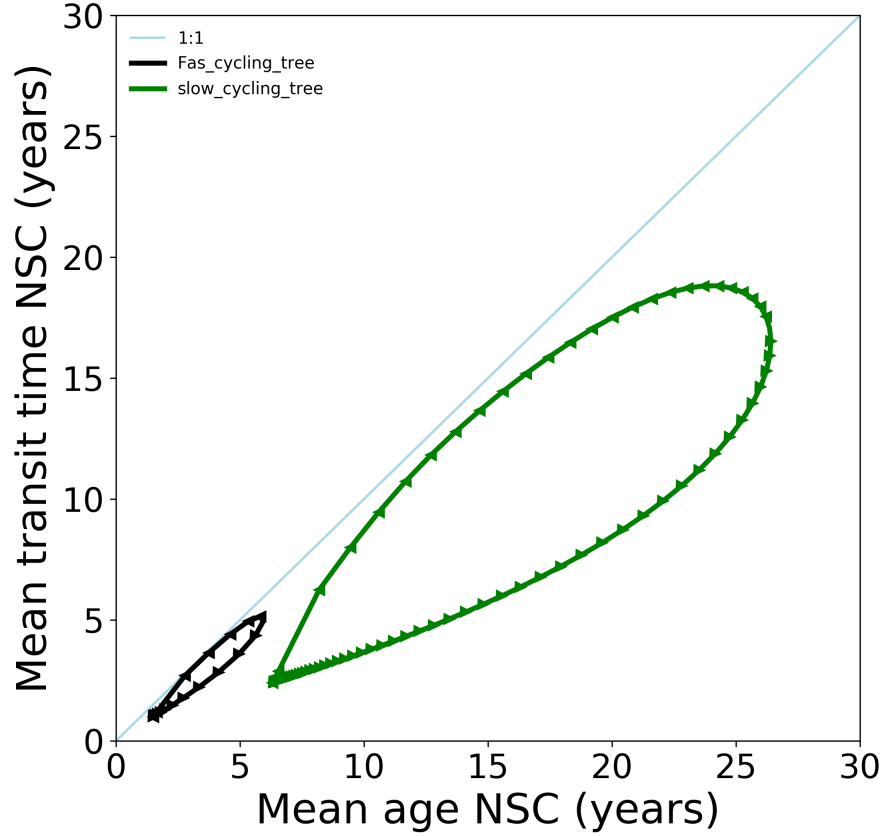


Figure 5: Simulation of the pathway that the metabolic safety margin of two trees with contrasting NSC turnover times would follow after facing a reduction in photosynthesis. The black line represents a tree with a fast turnover time, while the green line represents a tree with a slower turnover time. The arrows in the line show the direction of the changes at each time steps. Each arrow represent one time step (1 year) in the simulation.

generally have very young NSC because they cycle it daily, while stemwood and coarse roots would have an age composition with larger proportions of older carbon because their NSC cycling would reflect multiannual dynamics. Then, if metabolic safety margins would inform us about how well prepare are trees for future uncertain conditions at multiannual scales, mean NSC ages and transit time of long term reserves such as the ones in stem wood and roots would give us a good estimate of the metabolic state of trees. Stem wood reserves can serve as an integrator of carbon dynamics in the whole tree. ^{14}C mean ages of NSC have been reported to be older in stem wood and coarse roots than in any other tree organ. These ages show a large variability depending on the tree species and environmental conditions. It has been shown that trees access these reserves to support metabolism during girdling experiments, where the ^{14}C age of the CO_2 coming from the stem wood became progressively older with time while trees use their stored NSC. Trees also used their carbon reserves to rebuild new tissues in order to recover after a catastrophic event, where ^{14}C ages of the carbon use to build new

branches and roots resemble the ages in the NSC pools of the stem wood and coarse roots. Therefore, measuring the ^{14}C in the NSC reserves and respired CO_2 in the stem wood would can be use to have a good proxy of the metabolic safety margin of the entire tree for mature trees.

3.2.2 What compounds should we measure?

As stated before, NSC is mainly soluble sugars, starch and neutral lipids. They all serve as storage reserves, but they may have different physiological functions as well. Soluble sugars would also be involved in osmoregulation, cell signalling and communication, and can act as hormones, which are vital function for trees that need to be maintain. Neutral lipids may be involved in defense, stress responses, hormone signaling pathways, and cellular transport, as well. Therefore, there may be a proportion of soluble sugars and neutral lipids that may not be accessible to support metabolism and growth in mature trees.

Starch seems to have the solely function of carbon and energy storage, which is accessible to support other metabolic and physiological functions. There are still uncertainties about the accessibility of all the starch reserves for supporting trees metabolism. Nevertheless, there is evidence that trees can remobilize almost completely all starch reserves in the leaves and in the stemwood, at different scales and in response to perturbations. This may indicate that most of the starch pools may be entirely accessible to support metabolism during stressful conditions or for recovering from perturbations.

Estimating the age of the starch using ^{14}C could be the best proxy of the mean age of the NSC to estimate the metabolic safety margins in mature trees. Starch dynamics may be a metabolic integrator of the trees carbon dynamics, and it can serve as a metabolic integrator of the whole tree metabolism. It is possible to isolate starch for ^{14}C using enzymatic extraction methods, paying special attention in avoiding contaminations with exogenous carbon. Alternatively, estimating the age of the soluble carbon using ^{14}C may be a viable alternative. Soluble carbon is easier to extract than starch, although it may not represent 100% of the accessible reserves, if we assume a fast interchange between all soluble carbon (sugars, proteins, tannins, etc) and starch reserves in the sampled organ, the age of soluble carbon would also be a reliable representation of the NSC mean age. Here, we propose the use of ^{14}C measurements of starch as the best proxy for the NSC mean age of the storage compartments, but the use of soluble carbon may also give valuable information in case of lacking the technical capacity for starch isolation.

To estimate the metabolic safety margin we still need to estimate the NSC transit time. As stated before, NSC transit time is the carbon allocated to metabolism (mainly respiration and growth). If we assume that cell respiration is a priority over growth. Then changes in the age composition of the emitted CO_2 would be more sensitive to changes in the metabolic conditions of trees following stress or perturbations than the age composition of carbon allocated to growth. Therefore ^{14}C of the respired CO_2 would be a good proxy of the transit time of the accessible NSC stored in any tree organ. We hypothesize that, metabolic safety margin for mature trees would be more informative if the mean age of the NSC and mean transit times are estimated from the stored starch pools and the respired CO_2 , respectively, in the stem wood or roots.

3.2.3 Some examples

We estimated the metabolic safety margin measured at different organs to test our hypotheses in different sets of trees.

We did these measurements on tropical trees growing at a seasonal dry forest in Mato Grosso, Brazil. We measured species with contrasting storage traits (starch storage in

the parenchyma cells and starch storage in the living fibers) to evaluate if differences in the prioritization to storage can be detected in the metabolic safety margins. We also measured during the wet and the dry season to evaluate the impact of seasonal stressful conditions on the metabolic safety margin. Additionally, we measured these quantities in the branches and roots of *Larix decidua* and *Pinus mugo* growing in the Swiss Alps to evaluate the representativity of the storage organ in the metabolic safety margin.

Estimates of the NSC mean age were done based on ^{14}C measurements of soluble carbon extracts from stem wood cores following the methods described in Hillman et al... Estimates of the CO_2 ages were done based on ^{14}C measurements of the CO_2 incubated from stem wood cores following the methods described in mthur et al, hilman et al and pltier et al. The incubations were done for 36 hours to estimate the age of the carbon allocated to respiration at the moment of sample collection, avoiding remobilization of older, long-term, reserves.

Our measurements show that there are differences in the metabolic safety margin measured in the stem wood between storage strategies for the tropical trees in the Amazon (Fig. 6). Fiber storing species, which prioritize allocation to NSC, have lower metabolic safety margins than parenchyma storing species (Fig. 6), where most of the trees are far away from the 1:1 line in Figure 6. Seasonality in precipitation also seems to have an effect on the metabolic safety margin, reflecting the impact of the dry season on trees metabolism and use of storage reserves. During the dry season (July 2018) the metabolic safety margin was closer to the 1:1 line for most of the measured trees, while during the end of the wet season (May 2019) all trees show a recovery of the carbon reserves reflected in younger mean ages and younger mean transit times (Fig. 6).

We observed differences in the metabolic safety margins estimated from different tree organs. In our measurements in *L. decidua* and *P. mugo*, branches show younger mean ages and transit times than 1-2mm roots (Fig. 7), indicating an apparent higher turnover time in NSC in the branches than in the roots, which is in agreement with previous studies. Furthermore, differences in the metabolic safety margin between species can be observed only for root samples (Fig. 7), which store older reserves and may be better reflecting long term metabolic dynamics of trees. This supports our hypothesis that evaluating metabolic safety margins in roots and stem-wood would give more information about the metabolic state of the tree than organs with faster turnover times.

3.2.4 Important technical considerations about sampling CO_2 to estimate NSC transit time

Tissue specific incubations may be the most efficient way to collect CO_2 to estimate NSC transit time for the tissues of interest. When sampling for the stemwood or roots, some considerations should be taken:

1. Respiration should be obtained only from the living wood core, phloem and heartwood should be excluded. Sometimes when it is difficult to determine where the sapwood ends, it would be useful to use lugol iodine in the wood core to identify the proportion of living wood to be incubated. Incubating heartwood may influence the ^{14}C measurements because it would also reflect the respiration of other microorganisms that are feeding from the structural carbon in the heartwood. Decomposition of heartwood can generate not only CO_2 coming from the microorganisms in the wood but it would also collect methane CH_4 that would contaminate ^{14}C measurements of the collected material and would alter the estimations of NSC transit time.

In the same sense, incubated sapwood should not be extremely infected by fungi or bacteria. If the level of infection is high then the collected CO_2 may mainly reflect

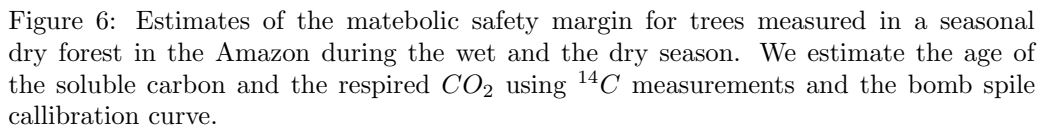
the respiration of microorganism and not wood respiration.

2. CO_2 easily dissolves in the sap and it is transported vertically in trees. Incubation of woody tissues immediately after collection would collect CO_2 that has not been respired directly by the incubated tissue, but CO_2 that was respired somewhere else and transported in the sap. After collecting the woody tissue, it should be left to stabilize with the ambient air for around 1 hour before incubation starts, in order to avoid contamination from transported CO_2 .
3. Wood or roots are living tissues that after collection would eventually start dying. There is a lot of uncertainty when living cells in the wood or roots would start dying after being excised from the tree, and what would cause their mortality. Nevertheless, we believe that extreme heat and dryness will contribute to accelerate dying of wood and root cells once collected. We recommend that after collection, tissues should be kept humid and in a fresh place to avoid premature death.
4. For incubations to reflect the transit time of the NSC in the stem wood or roots, they should not last long, the shorter the better, in order to avoid remobilization of the wood core storage reserves that would not be accessed if the wood core would have remained in the tree. Incubating tissues for many hours would result in the collection of the CO_2 corresponding to all the available reserves, as long as the wood tissue remains alive and respiration from growing fungi and bacteria remains negligible. The goal is to collect enough CO_2 to measure ^{14}C in the shortest time possible, and this would depend on the respiration rates and the quantity of the incubated living tissue. Nevertheless, we recommend incubation not to last more than 48 hours. This threshold would avoid remobilization of storage reserves that were not being used by trees at the moment of sampling, and the influence of microorganisms that would start growing in the tissue after a couple of days of the incubation.

3.3 Utility of the new hypothesis

Here we presented a new theoretic framework to evaluate the metabolic vulnerability of trees to future frequent and severe stress or perturbations. This framework helps us evaluate and understand the metabolic status of mature trees at a given time point, integrating the life history traits and storage dynamics of trees. From this metabolic state it can be estimated the potential turnover times of the accessible NSC reserves which influence the time that trees can rely on those reserves to survive and recover from stress or perturbations.

This theory also has the potential to help us understand patterns of recovery and adaptation in adult trees. Evaluating how NSC turnover times change in trees in response to stress and perturbation, measuring changes in the metabolic safety margin, will help us understand how NSC dynamics are affected by changes in environmental conditions and to assess the capacity of trees to recover from those changes. Information regarding the accessibility of NSC reserves and changes in NSC turnover times in response to environmental changes is still scarce. Our proposed framework offers a viable alternative to evaluate how apparent NSC turnover times would change when trees are exposed to environmental changes or have to face multiple perturbations.



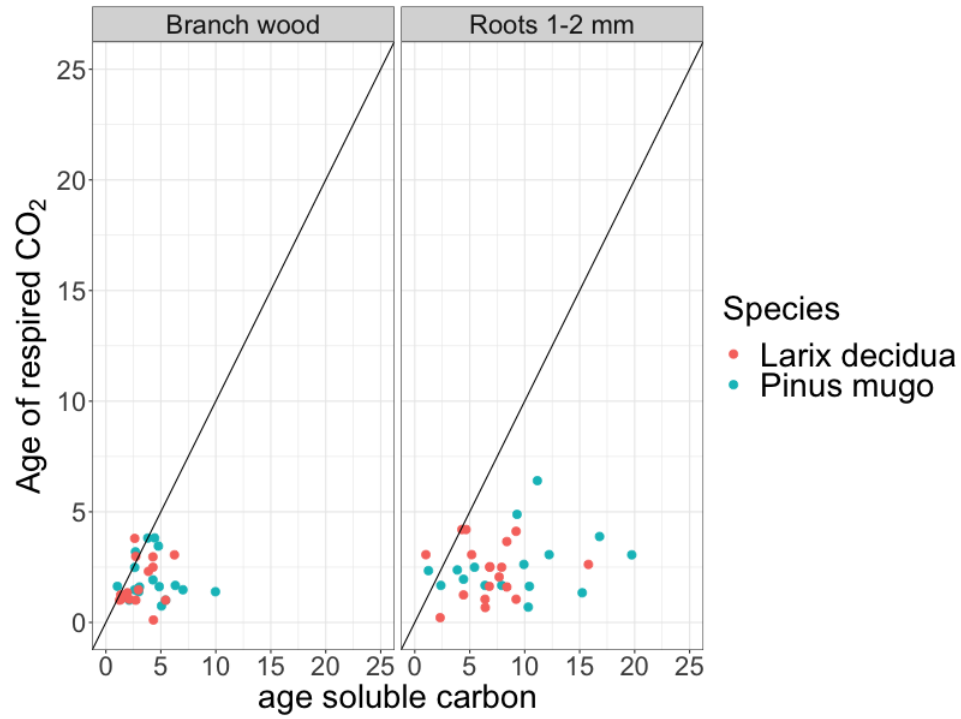


Figure 7: Estimates of the metabolic safety margin for *Larix decidua* and *Pinus mugo* in the swiss alps. We measured branches and roots from the same individuals. We estimate the age of the soluble carbon and the respired CO_2 using ^{14}C measurements and the bomb spike calibration curve.