Project Summary

The project goals are (1) to improve an existing photosynthesis vs. plant hydraulics trade-off model for plant gas exchange, (2) to test the model with greenhouse and growth chamber experiments, and (3) to apply the model towards more accurate predictions of forest health and tree mortality under climate change. The photosynthesis vs. plant hydraulics trade-off model takes relative photosynthesis gain as opportunity and relative hydraulic integrity loss as risk, and uses the instantaneous maximum of photosynthetic opportunity minus hydraulic risk to define how stomatal conductance should be regulated. The trade-off model provides a fundamental framework of stomatal regulation that predicts reasonable responses to environmental factors; however, it requires further developments and testing. Further developments of the model include post-drought plant physiology, optimization of energy allocation in root, stem, and leaves under stable environment, allocation shift strategy under varying environment. Theoretical analyses will be done on post-drought physiology based on xylem refilling, and adjustments of photosynthetic capacity and leaf area. Cost-benefit analysis will be used to predict optimal energy allocation under stable environment and the shift strategy during or after a drought. The model will be tested against measured plant response curves to atmospheric CO_2 concentration, vapor pressure deficit, and soil moisture in a growth chamber. Model extensions to post-drought physiology, energy allocation, and allocation shift strategy will be tested as well. To predict forest health under climate change, the model will be driven with projected hydrology and climate to simulate a productivity deficit as a function of various drought indices. The correlations between simulated productivity deficit and actual productivity as well as mortality will be deduced from hind-cast simulations on real stands where tree rings and survivorship data are available. The model will be used to produce species-specific maps of vulnerability and mortality as a function of drought index.

Intellectual merits

This study combines a new optimization theory of the photosynthetic versus hydraulic trade-off with the practical goal of predicting climate change impacts on forest health. Advances include applications of post-drought physiology and optimal investments at the individual level, and forest health prediction and tree mortality at the forest-stand level. Additionally, this study will also be important for future research in plant adaptations and acclimations to environment and can help resolve the species distribution pattern and growth pattern theoretically. This study would promote the integration of plant hydraulics into bigger models and hence to help predict how plant respond to environmental changes more accurately at regional or even global scale.

Broader impacts

The result of this study will be reported by scientific publications, conferences, and department seminars to reach broad audience. We will engage in promoting people to take the CMH into land-surface models to help better stomatal control scheme, as well as in exploring the model with deeper sights to help resolving more details in plant economy. Land managers will be informed with the model predicted tree mortality and be instructed with how to utilize the water resource to obtain best control of forests. I will also use PyQt to construct a GUI program that helps the public to visualize the project results, such as how plants become with different precipitation inputs, how plants drop the leaves when being stressed, and how forest health change with climate change; and through the program the public would get better understanding how much global change will alter our living environment.

Project Description

BACKGROUND

During the past 60 years, atmospheric CO_2 concentration has increased from 314 to 406 ppm (parts per million); and the accumulation of the greenhouse gas has led to 0.95 ℃ increase of mean annual temperature over this time period (NASA GISS). The rapid temperature increase (global warming) results in unprecedented global climate change. Global warming has already caused unpredicted drought stress and tree mortality across the globe (McDowell et al., 2015; McDowell and Allen, 2015; Anderegg et al., 2016). To evaluate and predict the impacts of global warming on forest health, tremendous effort has been devoted to modeling the global water and carbon cycles. However, the models used are still far from satisfactory, especially when dealing with plant hydraulic traits (Powell et al., 2013; Anderegg et al., 2015; Anderegg et al., 2016). Key to successful modeling of water and carbon cycles is the stomata on leaves, as leaves are where gas exchange happens. Leaf conductance to water vapor (G_H) and to CO_2 (G_C) are usually dynamically regulated via stomata in response to environmental factors, e.g. atmospheric vapor pressure deficit (VPD), soil moisture, light, and atmospheric CO_2 concentration ([CO_2]). However, the complexity of stomatal control has been a great challenge for modelers due to the lack of knowledge in underlying principles.

One practical approach is to deduce empirical rules from historical observations. Ball *et al.* (1987) established an empirical model based on experimental observations, where $G_H = k \cdot A \cdot h_s / c_s$ (k is a coefficient, A is the photosynthetic rate, h_s and c_s are humidity and CO_2 mole fraction difference across the stoma). This empirical model was further improved by applying more practical functions of humidity and CO_2 gradient (Leuning, 1995; Medlyn et al., 2011). Although reasonable responses to VPD and [CO_2] can be generated, these curve-fitting based models lose their predictive power when dealing with different data sets, especially when soil moisture is low (Powell et al., 2013; Buckley et al., 2016). To track the G_H decline under decreasing soil moisture, the empirical functions are multiplied by a phenomenological β function that falls from 1 to 0 as soil dries out; and the adoption of β function has helped capture both the seasonal and diurnal dynamics of stomatal control according to Powell *et al.* (2013). The phenomenological β function was not, however, physiologically based and therefore became another exercise in curve fitting.

Another option is to model the stomatal control mechanistically. A stoma is formed of two guard cells, and the aperture of the stoma is a direct result of the guard cells shape. When guard cells are hydrated, the turgor pressure of the guard cells is high and pushes the stoma open; when guard cells are dehydrated, the turgor pressure of the guard cells is low and pulls the stoma shut. The turgor pressure can be passively controlled by the water supply and be actively controlled by the in-cell osmotic concentration. The passive control is also known also as feedback control by the guard cell water potential (Cowan and Farquhar, 1977), and this explains the more stomatal closure under drier air or soil. The active control is able to respond to light and ABA, wherein blue light stimulates stomatal opening and ABA signals stomatal closure (Lovisolo et al., 2008; Martorell et al., 2014). In addition, stomata are able to sense atmospheric [CO_2] as they shut more under elevated [CO_2] (Medlyn et al., 2011). However, the mechanism of how light, ABA, and [CO_2] trigger the stomatal control quantitatively remains unsettled. Moreover, the correlation between stomatal adjustment and guard cell turgor pressure is not defined either, and localized dehydration makes the problem even more complicated (Tyree and

Yianoulis, 1980; Yianoulis and Tyree, 1984). Despite the mechanistic modeling approach may offer insights into how stomata are controlled, it is still far from being practical in ecological studies (Buckley and Mott, 2013).

Potentially the most powerful modeling approach is to derive the goal-oriented solution for stomatal behavior, e.g. assuming that plants are optimizing water use relative to photosynthetic gain. Two extremes of the water usage strategy are using no water and using as much water as fully open stomata allow; the former choice leads to no cost in water but no gain in carbon fixation while the later choice maximizes carbon gain but risks killing the plant by desiccation. Therefore, plants must be able to utilize the water in an intermediate way that maximizes the overall profit. Such optimal water usage would drive the plants to close stomata more when CO_2 resource is relatively more abundant and when air or soil is drier. These expected responses for a goal-oriented solution agree qualitatively with experimental observations (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007), suggesting the utility of an optimization approach for modeling gas exchange. The advantage of the goal-oriented solution is that we do not need to know all the underlying mechanisms of how stomata can be regulated quantitatively or to make any empirical fittings. The only concern is how we define the optimum criterion. Two major hypotheses are the Water Use Efficiency Hypothesis (WUEH, Cowan and Farquhar, 1977) and Carbon Maximization Hypothesis (CMH, Wolf *et al.*, 2016).

According to WUEH, plants should maximize the photosynthetic gain for a given amount of water used during a given time period (Cowan and Farquhar, 1977). This is a "constrained-optimization" problem without an exact solution. The WUEH solution specifies a constant Lagrangian multiplier $\lambda = dE/dA$, meaning stomata act to maintain a constant dE/dA over the optimization period. The optimal solution will change according to the water availability and time even though other environmental cues stay constant. For instance, if twice the amount of water is available during the same time period, the same λ will not meet the WUEH criterion. A constant λ predicts reasonable responses to VPD, and WUEH has been successful in explaining the empirical data from many species and environments where [CO_2] and soil moisture are stable. However, constant λ predicts an inconsistent trend in response to [CO_2] and no change to soil moisture. Moreover, WUEH tracks the water usage in a given time period, and allows plant to save water in wet soil and use that as soil dries out so as to maintain a constant λ; but the fact is the saved water can be ‘stolen’ by competing species. Thus, it can be argued that constant λ applies only to the scenarios without competition for water. To summarize, the problems with WUEH are (1) arbitrary water and time has to be set at the first place to make λ a useful parameter, (2) λ cannot be derived from the model directly and it can only be deduced from data fitting (Buckley et al., 2016), (3) λ is not able to capture the right trend to [CO_2] and soil moisture, (4) constant λ is not realistic as water saved by WUEH plants can be stolen by competing plants.

According to CMH (Wolf et al., 2016), at every instant time, plants are supposed to maximize the difference between the instantaneous opportunity for photosynthetic gain (B) and the instantaneous risk associated with hydraulic failure (Θ). The solution of CMH is when B-Θ is maximized, i.e. when $dB/dG_H=d\Theta/dG_H$ or $dB/d\Theta=1$. In CMH, plants ought to adjust the stomata aperture to maintain $dB/d\Theta$ at 1. The advantages of CMH are (1) model is supposed to respond to instantaneous environmental cues and therefore no water amount or time is arbitrarily constrained as in WUEH, (2) predictions can be made simply with knowing the basic plant hydraulic and photosynthetic parameters and the environmental cues, thus no empirical fitting of coefficients is required (3) reasonable trends to all environmental cues can be generated, and (4) the optimization criterion is ecologically realistic as plants will use water more aggressively when photosynthetic opportunity is high or hydraulic risk is low.

The proposed research aims to develop and test the CMH concept, and to predict stomatal behavior and forest health in response to environmental cues. To implement CMH, we need to quantify the B and Θ. The B can be obtained from standard model of photosynthetic capacity (Farquhar and Sharkey, 1982). The Θ, which is the risk in plant hydraulic function to transport water, requires quantifying the risks in a series of transporting elements from soil to leaves. Water transportation is driven by a positive pressure difference from soil to leaves, and the pressure gradient is usually achieved by negative pressure in leaves generated by capillary force. Due to gravity and resistance to water flow, the water pressure drops along the flowing water column and gets most negative at the downstream end. The meta-stable water column under negative pressure is protected from air entry by the conduit pit membranes; but once the pressure difference (between water and air) across the pit membrane is higher than a threshold, the water column breaks leading to cavitation of the conduit (Sperry and Tyree, 1988; Tyree and Ewers, 1991). The conduit cavitation in xylem results in a decline in water transportation capability as part of the flow pathway is blocked by air bubbles. A vulnerability curve is the plot of hydraulic conductance of an element versus the applied water pressure (P) in the element. The conductance starts high when xylem pressure is atmospheric (P set to 0) or above, and decreases and eventually vanishes as water pressure becomes sub-atmospheric (Tyree and Sperry, 1989; Tyree and Zimmermann, 2002). When water flow rate increases, more negative downstream pressure is required, and hence more decline in whole tree hydraulic conductance. As a result, there is a maximal limit to transpiration, E_{crit}, beyond which the most downstream end fails to transport water completely because the water pressure would be too negative (Sperry et al., 1998).

The risk of stomata opening, Θ, is derived from the hydraulic limitation on the transpiration rate (E). At a given soil water pressure (P_{soil}), as stomata open more and more, E increases from 0 to E_{crit}, and the downstream canopy leaf water pressure (P_{canopy}) decreases from P_{soil} to P_{crit}, where E_{crit} is achieved. The plot of E versus P_{canopy} is the water supply curve, which indicates the water transport capability of the plant (Sperry and Love, 2015; Sperry et al., 2016b). The supply curve is shaped by the vulnerability curves of all the transporting elements (including that of the soil) and P_{soil}. Following the supply curve, the slope, i.e. dE/dP, represents the limiting hydraulic conductance at the downstream end of the flow path where P is most negative (P_{canopy}). The dE/dP drops from its maximal at $P_{\text{canopy}} = P_{\text{soil}}$ (no additional risk to plant) to 0 at $P_{\text{canopy}} = P_{\text{crit}}$ (plant desiccation). Therefore, we use dE/dP as an indicator of hydraulic risk, and the risk function is defined and normalized as $\Theta = 1 - k/k_{\text{max}}'$, where k and k_{max}' stand for the dE/dP at target P_{canopy} and P_{soil}, respectively. The dimensionless normalized risk rises from 0 (no risk) to 1(maximal risk that results in desiccation) as P_{canopy} goes lower (or E goes higher). Examples of hydraulic loss and relative risk can be found in Figure 1 (dash-dotted lines).

The opportunity for photosynthetic gain, B, also depends on P_{canopy} because higher stomatal opening promotes a higher photosynthesis rate. Following the same supply curve, the target E can be translated to G_{H} and therefore photosynthesis rate. Briefly, at the given P_{canopy}, canopy transpiration rate, E, is gained from the supply curve. Leaf specific transpiration is used in leaf energy balance in order to calculate leaf temperature (Campbell and Norman, 1998). Then the leaf-to-atmosphere VPD is calculated from leaf temperature and atmosphere humidity. G_{H} and G_{C} are then computed from leaf specific transpiration and leaf-to-atmosphere VPD as G_{H} =

E / VPD and $G_C = G_H / 1.6$. G_C and atmospheric [CO_2] are used to obtain photosynthesis rate (A) and inter-cellular CO_2 concentration (C_i) from a modeled $A \sim C_i$ curve. The normalized B is defined as the photosynthetic rate, A, over the maximal photosynthetic rate (A_{max}') that can be achieved at the given soil water potential, i.e. B = A/A_{max}'. The dimensionless normalized opportunity ranges from 0 (no opportunity) to 1(maximal opportunity, usually at P_{crit}) as P goes lower (or E goes higher). Examples of photosynthesis rate and relative opportunity can be found in Figure 1 (dashed curves).

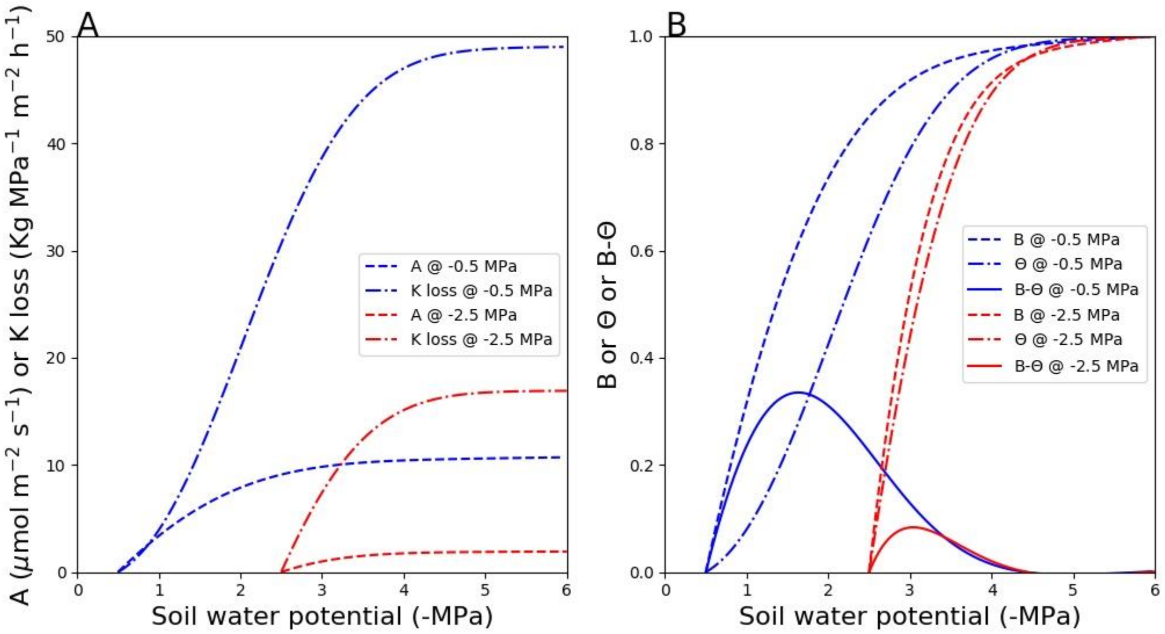

Figure 1. Examples of CMH optimization at different soil moisture. Panel A shows the photosynthesis rate and loss in plant hydraulic conductivity along the supply curves at -0.5 and -2.5 MPa. Panel B plots the relative opportunity (B), risk (Θ), and opportunity – risk (B – Θ) at -0.5 and -2.5 MPa

The CMH optima is the maximized instantaneous B – Θ, namely $A/A_{max}' + k/k_{max}' - 1$. The profit is maximized when $d(A/A_{max}')/dP + d(k/k_{max}')/dP = 0$, or $d(A/A_{max}')/dG_H + d(k/k_{max}')/dG_H = 0$. At an instant in time, optimal stomatal behavior is obtained in response to environmental cues; and optimal stomatal behavior changes with environmental cues, such as soil moisture. Examples of CMH optima (both absolute and relative opportunity and risk) can be found in Figure 1. As presented in Sperry *et al.* (2016a), CMH predicts reasonable trends for all environmental responses to atmospheric humidity, soil water potential, light, and atmospheric [CO_2]. The CMH concept of photosynthesis versus plant hydraulics trade-off makes it possible to explicitly predict the stomatal behavior at any given environment without employing data fitting.

Our CMH model (Sperry et al., 2016a) was inspired by Wolf *et al.* (2016) model, which is another case of CMH. Wolf *et al.* (2016) put up the idea that an evolutionary stable strategy ought to drive the stomatal control to optimize instantaneous carbon gain with pricing the instantaneous direct carbon cost of xylem dysfunction, including the cost of embolism refilling. However, the quantitative correlation between the cost of xylem dysfunction and energy consumption in carbon unit is unknown and probably varies according to xylem water potential and species. Our CMH risk formulation differs from Wolf *et al.* model by specifying the risk in hydraulic integrity as the penalty of stomatal opening rather than an instantaneous carbon cost that is impossible to quantify with our current understanding of plant hydraulics. In engineering terms, the water transportation system is analogous to an engine, with higher transpiration rates leading to engine overload. The Wolf et al. model focuses on the cost of engine damage and repair, whereas our interpretation is based on maintaining a safety factor against engine failure. The general idea of the two versions of CMH is the same, i.e. plants are increasingly penalized as they go further along the supply curve. If we express our CMH risk function in carbon units, the risk scales with the instantaneous maximal photosynthetic rate: $\delta\Theta = \delta k \cdot A_{\max}/k'_{\max}$. In steady state models, this makes sense because the instantaneous value of the tree is its maximal potential photosynthetic gain at that instant; and the risk should scale with the instantaneous value.

Despite the potential for predicting stomatal response to environmental cues, CMH has not yet been tested and requires more developments. The developments include post-drought physiology and plant investment adjustments in order to implement the short-term and long-term physiological changes in plants. What happens after a drought may impact the CMH predictions as B and Θ functions may be altered, such as xylem refilling strategy, photosynthetic capacity change, and leaf area change. These are often observed on plants after a drought (Brodersen et al., 2010; Zhou et al., 2014; Charrier et al., 2016; Wolfe et al., 2016; Zhou et al., 2016). The abscisic acid (ABA) metabolism further makes the post-drought stomatal regulation more complicated as ABA signals the plants to down-regulate the stomatal conductance (Lovisolo et al., 2008; Melcher et al., 2012; Brodribb and McAdam, 2013). Experimental observations showed insignificant change in leaf water pressure and down-regulated stomatal conductance after plants return to unstressed scenarios from drought. The observations can be explained by either hard-wired risk function or combined effects from xylem refilling, photosynthetic capacity change, and ABA metabolism. Thus, CMH needs to be extended to account for these complicated scenarios in order to make better predictions after drought.

The CMH and the post-drought implementation focus on the short-term response to environmental factors based on the known parameters under steady state, such as fixed allocation to leaf area and photosynthetic capacity. In reality, plants are able to adjust the energy allocation in different tissues in order to optimize long-term productivity. Therefore, CMH needs to be extended to predict long-term energy allocation shifts. If CMH fails to implement the optimal energy allocation, the fixed parameters will allow reliable predictions on gas exchange and hence forest health in long-term simulations; and this is the same problem that empirical models suffer from.

While the model and the developments are initially focused on an individual plant scale, the model will be expanded into forest stand scale to make predictions of forest health. The potential of CMH in dealing with both photosynthesis and plant hydraulics makes it promising in future research in predicting forest health. The extra interface to plant hydraulics and soil profiles of CMH also makes it possible for land-surface models to deal with soil and plant water relations more sophisticatedly.

AIMS

The objects of this research are to explore the CMH in several aspects, to test the model at the whole-plant level, and to apply it to predict forest health at the forest-stand level. The CMH will be programmed to run simulations of how plants respond to environmental cues, how plants optimize the investments in different tissues, and how climate change affects tree mortality and forest health. The model will be tested in growth chamber, greenhouse, and field stands. Further knowledge on how tree mortality and forest health are correlated to drought stress will be deduced with historical data from real forest stands. The correlations will be applied to model simulations, and predictions on forest health as well as individual species mortality will be examined as a function of drought severity.

Aim 1: Improve the CMH

The primary goal of aim 1 is to build a model to accurately predict plant responses (gas exchange, productivity, and mortality proxies) to environmental cues in both short and long term. For short term, simulating accurate opportunity and risk functions is essential, and for long term, tracking physiological trait changes is necessary. I propose to improve our current CMH in the following respects: (1) post-drought performance, (2) optimal root-stem-leaf allocation for constant conditions, and (3) investment adjustment strategy in the presence of drought.

(1) Post-drought performance

Drought may result in persistent xylem cavitation, hence potentially altering the hydraulic risk function and the CMH optimum. Xylem cavitation occurs when conduits are exposed to low water pressures associated with drought. In order to maintain hydraulic function after a drought is over, surviving trees may or may not refill the cavitated conduits and grow new conduits. In the very short time after drought, new xylem growth would not have great impact on the hydraulic function; thus it is critical to know how the cavitated conduits affect the post-drought CMH optimum. According to CMH, the risk of opening stomata, Θ, is the decrease in hydraulic safety margin. On assuming rapid cavitation refilling and unchanged photosynthetic capacity, there will be no change in Θ and B functions or the CMH optimum. However, if xylem cavitation persists, Θ would be stuck at zero as long as leaf water pressure is higher than that during the drought, because there would be no new cavitation over this pressure range. Then the CMH optimum will be pushed towards a low water pressure same as that during the drought (Figure 2, with cavitation plots). This unrealistic behavior suggests that if cavitation is not reversed, the risk function should nevertheless stay unchanged, as if it is "hardwired" to the un-cavitated supply function (Figure 2, hard-wired plots).

Meanwhile, any persistent change in photosynthetic capacity (i.e. maximal carboxylation rate $V_{c,max}$ and maximal electron transport J_{max}) and leaf area during the drought would have continuing impacts on the post-drought CMH optimum by altering the B function. Reductions in photosynthetic capacity can happen in both short-term and long-term drought, whereas the change in leaf area might only happen during long-term drought. Drought also causes higher concentrations of leaf abscisic acid (ABA) which signals stomatal closure. If high ABA persists after the drought, it could prevent the leaf from achieving the CMH optima (an ABA "after-effect"), at least until the ABA is degraded. The experimental observations of reduced transpiration and insignificant P_{canopy} change may also be explained by the altered CMH optimum from historical optimum via changes in photosynthetic capacity and leaf area or an ABA after-effect (red arrow in Figure 2C). Therefore, we need to determine whether the risk function is hardwired to the un-cavitated supply function and determine how an ABA after-effect would interfere with the attainment of the post-drought CMH optimum.

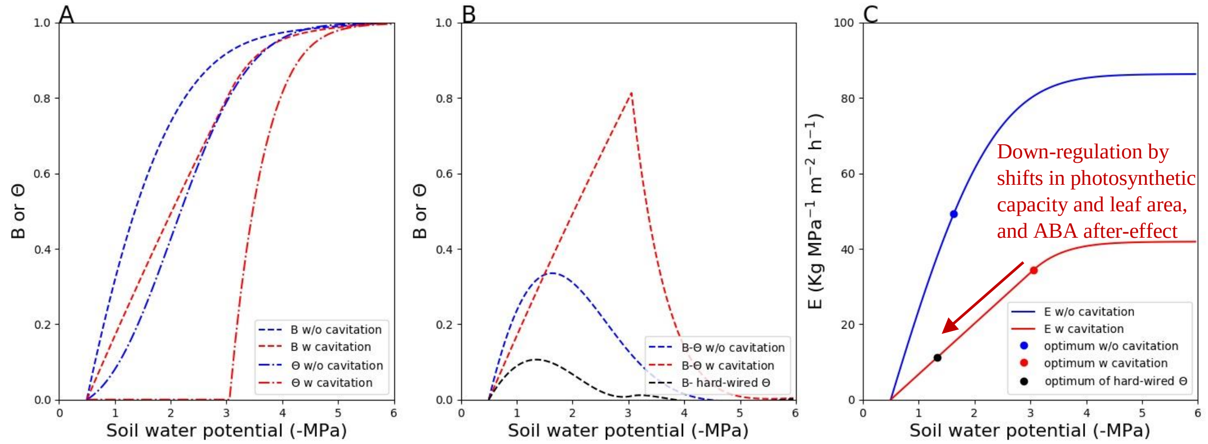

Figure 2. Examples of post-drought CMH trade-off. Panel A plots the B and Θ functions with and without cavitation. Panel B shows the three B – Θ curves originated from panel A. The blue curve represents the profit curve without cavitation (blue dashed – blue dash-dotted); the red curve plot that with cavitation (red dashed – red dash-dotted), and the black curve plot the profit of hard-wired Θ (red dashed – blue dash-dotted). The optimal transpiration rates of three optimum options are plotted on the supply curves without and with cavitation.

I propose to program the model to run simulations on all permutations of post-drought CMH solutions, including (1) moderate droughts (no leaf drop), (2) xylem refilling magnitude, (3) hardwired vs. cavitation-altered Θ function, (4) photosynthetic capacity changes, and (5) ABA after-effect. The default settings are no xylem refilling, hardwired risk function, unchanged photosynthetic capacity, and no ABA after-effect. **Predicted results**: different outputs from the permutations differ in P_{canopy}, transpiration rate, and photosynthesis rate; and the experimental observations on P_{canopy}, transpiration rate, photosynthesis rate, photosynthetic capacity changes, and xylem refilling during the drought-rewatering cycle allow the proper CMH optimum to be modeled; the ABA after-effect could be detected if plants consistently under-estimate the optimal transpiration rate (or stomatal conductance). **Potential pitfalls**: actual plant responses may not be consistent with either ABA after-effect or the predicted CMH optimum.

(2) Optimal root-stem-leaf allocation for constant conditions

Plants are able to control the energy allocation in different tissues in order to obtain greater fitness. For example, plants tend to have lower root-to-shoot ratios, higher leaf area, and higher photosynthetic capacity in rainforest compared to the desert plants (Sperry et al., 1998). The allocation includes shorter-term investments in leaves at a growth season scale and longer-term investments in root and stem at multi-year scale. Successful energy allocation allows plants to use water more efficiently in the growth season and acclimate to the environment in the long-term growth. While it is possible to measure the root-to-shoot ratio, leaf area, and photosynthetic capacity, great efforts have to be taken. Meanwhile, the parameters need to be measured periodically because the optimal allocation varies according to environment. Modelling the optimal allocation would provide quantitative understanding towards physiological acclimation to environment as well as save experimental efforts to measure the parameters.

The initial step towards modelling the optimal allocation will be to characterize the optimizations under constant environmental conditions, intending to represent the typical growth conditions in the stable habit. Plants ought to optimize the leaf area and photosynthetic capacity during the time of growth. Assuming fixed long-term investments in root and stem, growing a higher leaf area benefits the plants for more photosynthetic gain, but costs the plants more energy in leaf construction and leaf respiration. While the photosynthetic gain peaks with increasing leaf area, the construction and respiration costs increase proportionally to leaf area; therefore there must be an optimal leaf area for a given leaf-specific photosynthetic capacity ($V_{\mathrm{c,max}}$ and $J_{\max}$). Higher photosynthetic capacity also benefits plants by increasing photosynthetic rates but costs the plants more energy in leaf respiration. Provided that light and water resources are limited but respiration rate increases proportionally to photosynthetic capacity, there must be an optimal photosynthetic capacity as well as an optimal leaf area. Plants are supposed to maximize the profit associated with leaf investments, i.e. total photosynthetic gain minus the costs in leaf construction and leaf respiration. The optimal leaf area and photosynthetic capacity ought to vary according to the root and stem water transport capability, soil and atmospheric moisture, and light and carbon resources during the growth season. When water transport capability is lower, photosynthetic gain peaks at lower leaf area. When soil or atmosphere is drier, less water availability or higher vapor pressure gradient would push plants to use water more conservatively, and hence lower leaf area and photosynthetic capacity applies. When light and carbon resources are more abundant, higher leaf investments can be more profitable. Figure 3A and 3B plot the contour of carbon profit versus the varying $V_{\mathrm{c,max}}$ and leaf-to-bsal area ratio at wet and dry soil, respectively; the model simulations on constant wet and dry environmental conditions agree with the analyses above.

The above analyses on optimal leaf area assume a fixed allocation in root and stem. However, the partitioning of the investment between root and stem should also be optimized because it influences the water supply capability to leaves. If plants invest too much in the root, the low stem hydraulic conductance would expose high hydraulic risk to the plant system resulting in lower profit, and verse versa. The optimal investment in root-to-stem ratio depends on the CMH trade-off which influences the stomatal behavior, and the price of the investment, i.e. price of new xylem growth. For example, the optimal root-to-stem ratio differs when root xylem growth is twice the price of stem xylem growth from that of same price, and plants would investment relatively less energy in stem (but relatively more stem biomass) when stem price is cheaper. With a given amount of total energy for stem and root growth, plants may allocate the energy with a ratio from 1:0 to 0:1, and root and stem biomasses are obtained based on the known prices. At each ratio, the optimal additional investment in leaves will be calculated (as shown in Figure 3) based on the calculated root and stem biomasses. In this way, the optimal allocation in root-stem-leaf can be obtained.

For the leaf investments, I propose to run the CMH under different combinations of leaf area and photosynthetic capacity under constant environmental conditions, like Figure 3. Useful information on optimal leaf investment could be obtained for different environment scenarios, such as hydraulic efficiency, soil moisture, atmospheric humidity, and light and carbon resources. For the optimal root-to-stem ratio, I propose to run the optimal CMH leaf investments under different root-to-stem ratios based on known allocation prices. **Predicted results**: higher leaf area and photosynthetic capacity under wetter environment when root-to-stem ratio is fixed; higher root-to-stem ratio under drier environment. **Potential pitfalls**: the theoretical analyses rely on these assumptions: (1) profit in carbon unit is the criterion of optimal investments, (2) construction cost of leaf can be simply expressed as a function of leaf area, (3) the price in energy allocation is fixed, and (4) environmental conditions are stable. Any inconsistency with the assumptions would lead to error in optimal energy allocation strategy.

(3) Investment adjustment strategy in the presence of drought

Although it is possible to compute optimal allocation theoretically based on reasonable assumptions [section (2) above], the optimums are meant for constant environmental conditions. However, using leaf investments as an example, a dynamically changing environment ought to change the optimal leaf investments in every single second. If plants alter the leaf area accordingly, the energy used for constructing the leaves could be tremendously higher than maintaining a constant leaf area. Therefore, plants may have to tolerate lower photosynthesis under wetter conditions and higher respiration under drier conditions to compensate for the leaf construction cost. The kinetics of photosynthetic capacity would cause delay of optimal photosynthesis unless the photosynthetic capacity changes instantly. Therefore, the optimal investments are actually optimums based on the varying environment rather than dynamic optimums at each instant, and there should be threshold for the shift in optimal allocation.

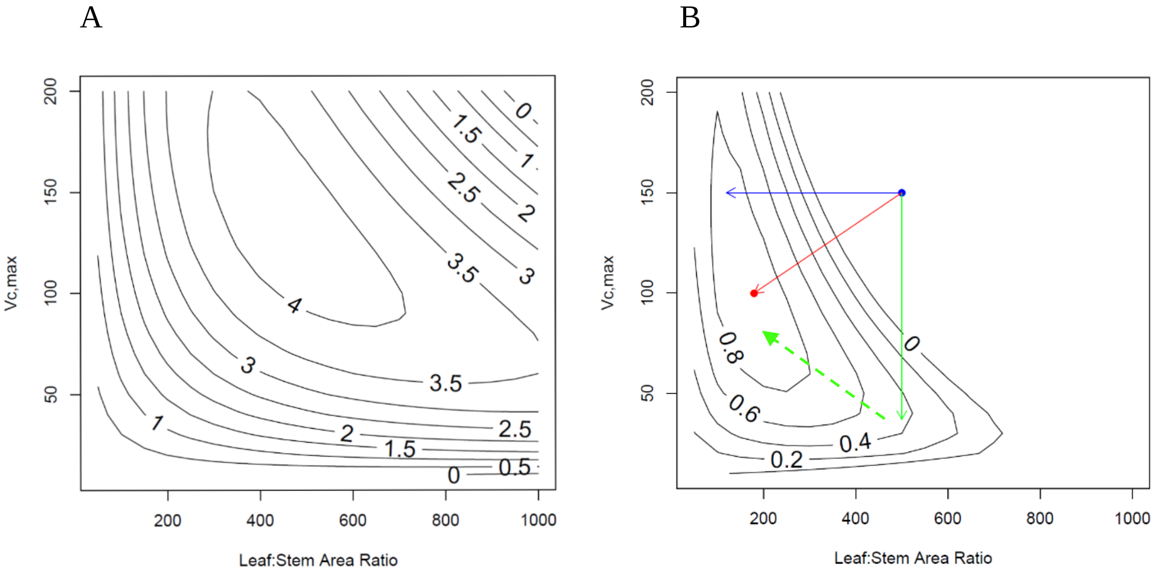

Figure 3. Examples of optimal investment into leaf area and $V_{c,max}$. A: optimal investment at soil water potential of 0.5 MPa; B: optimal investment at soil water potential of 1.5 MPa. The arrows in panel B indicate three possible options for the plants to obtain a higher profit.

When the environment becomes drier, plants tend to increase the root investment and decrease the leaf investments in order to maintain water supply capability to the leaves. Theoretically, the shifts of optimal physiological parameters depend on the timing of the changing environment. In a very short-term drought, plants might down-regulate the photosynthetic capacity to reduce the water consumption and respiration cost, but retain the leaves. In an intermediate-term drought, plants might shed the leaves as well to further decrease the costs. In a long-term chronic drought, plants might further change the root-stem-leaf ratios to optimize the water uptake and transport and hence maximize the profit. As CMH suggests optimal investments under stable environment scenarios, it could be beneficial for plants to adopt the optimal allocation when the climatic change lasts many years. However, it may not benefit plants much to change the root-to-shoot ratio in a few months of drought, to shed the leaves in a few days of drought, or even to down-regulate the photosynthetic capacity in a few hours of drought. In most cases, the optimal investment adjustments may not follow the optimum suggested by CMH model depending on the environmental change, like drought timing and frequency. Therefore, the problems in the investment adjustments are (1) the timing threshold for the adjustments and (2) the optimal adjustment magnitude. CMH predictions relies on the accuracy of physiological parameters, like hydraulic conductance of different tissues, the drought history of the tree, and the photosynthetic capacity. If CMH fails to track the shifts in the physiological parameters, it will not make reliable long-term predictions.

For the relatively short drought where root-to-stem ratio is not changing, optimal photosynthetic capacity would be achieved rapidly as soil dies down on assuming costless and rapid photosynthetic capacity adjustment. The arrows in Figure 3B show three possible ways for plants to get higher carbon profit in response to soil drying. The green arrow indicates the rapid change in photosynthetic capacity, which is efficient during a short-term drought; however, the carbon profit is much less than shedding leaves as other two arrows suggest. Plants may choose to shed the leaves all at once (blue arrow in Figure 3B) or shed the leaves gradually as drought continues (red arrow in Figure 3B). It is not beneficial for plants to shed the leaves at the first drought instant because it is unknown how long the drought will be. The reasonable strategies for plants are (1) shed leaves to maintain the optimal investment once a threshold is achieved (green dashed arrow in Figure 3B) and (2) change photosynthetic capacity and shed the leaves gradually as drought continues (red arrow in Figure 3B). If strategy (1) applies, a photosynthetic capacity increase is expected after the leaf shedding; if strategy (2) applies, gradual decrease in photosynthetic capacity should be observed along with leaf area change.

For the relatively long drought where root-to-stem ratio change is necessary, the progress can be slow because the energy comes from carbon profit gained during the growth season. When plants are stressed with drought and when plants are released from a fairly long drought, the root-to-stem ratio might change gradually and eventually reaches the optimal after years. Besides the timing and magnitude of adjustment, the pricing for new growth and xylem refilling can also be an issue with the optimal investments when xylem refilling is much cheaper than the new growth. Theoretical analyses of the optimal energy allocation strategy could improve our understanding in the long-term investments.

For the relatively short drought, I propose to use the CMH to run simulations on (1) optimal photosynthetic capacity change at different shorter-term droughts without leaf area adjustment; (2) optimal leaf area and photosynthetic capacity change at longer-term droughts; (3) optimal leaf shedding strategies under different drought length on assuming immediate leaf shedding at drought. For the relatively long drought, I propose to run simulations on (4) plants from wet condition to long-term drought and (5) plants from long drought to wet condition in order to understand the timing of energy allocation. **Predicted results**: experimental observations on leaf area and photosynthetic capacity change can be used to test the adjustment strategies in shorter and longer term drought; leaf shedding threshold or gradual leaf shedding strategies can be distinguished; it will take years for plants to obtain the optimal energy allocation during long-term drought and post-drought scenarios. **Potential pitfalls**: there could be significant cost in photosynthetic capacity adjustment, which is hard to quantify, and the cost may influence the CMH model accuracy; the price on new growth and xylem refilling is hard to quantify.

Aim 2: Test the CMH

Despite the potential in simulating stomatal behavior and tracking physiological changes, the CMH has yet to be tested. The accuracy of CMH including the trade-off criterion as well as extended perspectives listed in Aim 1 (i.e. post-drought performance, optimal investments, and investment adjustment strategy).

(0) Tree species and basic hydraulics measurements

Betula occidentalis (water birch) trees will be used to test the CMH. 120 trees will be grown in the greenhouse under wet environment. The testing experiments will be done in 2017. Trees will be divided into five groups, and the groups of trees are to (0) measure the basic hydraulic and photosynthetic parameters, (1) test fundamental short-term CMH responses to environmental cues, i.e. VPD, [CO$_2$], and P_{soil}, (2) test post-drought physiology, (3) test optimal investments strategy, and (4) test leaf investment adjustment strategies. For group (0), maximal hydraulic conductance and vulnerability curves (of root, stem, and leaf), leaf area per basal area, and photosynthetic capacity ($V_{\mathrm{c,max}}$ and J_{max}) will be measured.

(1) Test the fundamental short-term response

Short-term CMH responses to environmental cues will be tested in growth chamber where VPD, [CO$_2$] and temperature can be well controlled. Ten well watered trees will be moved into the growth chamber a week prior to conducting measurements. $A \sim C_{\mathrm{i}}$ curves of each tree will be measured after the acclimation to estimate the photosynthetic capacity ($V_{\mathrm{c,max}}$ and J_{max}). Then the plant response to varying [CO$_2$], atmospheric humidity, and soil moisture will be measured.

(a) [CO$_2$] response. Trees will be well watered the night before experiments, and bagged leaf samples of six bagged trees will be taken early in the morning to measure the predawn leaf water potential (P_{predawn}). Then light source will be set to 1000 µmol m^{-2} s^{-1}; air temperature set to 25 ºC; relative air humidity set to 60%; [CO$_2$] set in the sequence of 800, 600, 400 (ambient), 300, and 200 ppm. At each [CO$_2$] setting, plants will be hold in the environment for at least 1.5 hours before any measurement. The canopy leaf water potentials (P_{canopy}) will be then measured with leaf samples taken from the six trees at [CO$_2$] of 800, 400, and 200 ppm. At each [CO$_2$], photosynthesis rate (A_{leaf}), leaf stomatal conductance to water vapor (G_{leaf}), leaf temperature (T_{leaf}), and leaf-specific transpiration rate (E_{leaf}) will be measured with Photosynthesis system Li-Cor 6800; whole tree transpiration rate (E_{tree}) will be measured with a balance by weighting the tree every 10 seconds for 10 minutes. After measurements are done at each [CO$_2$], trees will be re-watered to prevent soil from drying.

(b) VPD response. Leaf samples from six trees are taken early in the morning to measure P_{predawn}. Light will be set to 1000 µmol m^{-2} s^{-1}; [CO$_2$] set to 400 ppm; air temperature set to 25 ºC. Relative humidity will be set in the sequence of 75%, 65%, 55%, 45% and 35%. After at least 1.5 hours' stabilization, P_{canopy}, A_{leaf}, G_{leaf}, T_{leaf}, E_{leaf}, and E_{tree} will be measured. Trees will be watered after each humidity gradient to maintain wet soil for later measurements.

(c) P_{soil} response. During the measurements, trees will be kept under 1000 µmol m^{-2} s^{-1}

light, 400 ppm [CO_2], 25 ºC air temperature, and 50% atmospheric humidity in the growth chamber. P_{canopy}, A_{leaf}, G_{leaf}, T_{leaf}, E_{leaf}, and E_{tree} will be measured everyday on six trees. After the measurements on each tree, the tree will be bagged and moved out of the growth chamber to stabilize for 2 hours; then leaf sample will be taken to measure the leaf water potential to use as P_{predawn}. After the measurement, the trees will be moved back to the growth chamber for further drought. Five P_{soil} gradients will be applied to each tree.

Leaf area and basal xylem area will be measured for the 10 trees after the response curves are finished. Measured hydraulic and photosynthetic parameters as well as leaf area and basal xylem area will be put into CMH model to run simulations of stomatal responses to environmental cues; model outputs will be compared to experimental observations to test the accuracy of CMH model. Leaf temperature will also be modeled and tested against experimental measurements, but experimental values will be used in the model to test the accuracy of the trade-off criterion. **Potential pitfalls**: leaf area impacts the transpiration rate and least leaf samples will be used; the P_{predawn} should be measured for each treatment in CO_2 and VPD response because of rapid soil drying in small pots, and the missing P_{predawn} may result in inconsistency between model and experiments.

(2) Test the post-drought performance

Six trees will be used to test the post-drought plant response. The experiment will be run under an individual tree for each drought-rewatering cycle. A tree will be moved to growth chamber 7 days prior to conducting experiments. Light will be set to 1000 µmol m^{-2} s^{-1}; [CO_2] set to 400 ppm; air temperature set to 25 ºC; relative humidity set to 50%. $A \sim C_{\mathrm{i}}$ curve, P_{predawn}, P_{canopy}, A_{leaf}, G_{leaf}, T_{leaf}, E_{leaf}, and E_{tree} will be measured prior to drought stress. Then tree will be dried for 2—3 days; in the progress of drought stress, $A \sim C_{\mathrm{i}}$ curve will be measured periodically and E_{tree} will be dynamically monitored. At the end of drought stress, $A \sim C_{\mathrm{i}}$ curve, P_{predawn}, P_{canopy}, A_{leaf}, G_{leaf}, T_{leaf}, E_{leaf}, and E_{tree} will be measured. Then the tree will be well watered and hold in a water reservoir to ensure abundant water resource. $A \sim C_{\mathrm{i}}$ curve, P_{predawn}, P_{canopy}, A_{leaf}, G_{leaf}, T_{leaf}, and E_{leaf} will be measured every day for 3—10 days after the re-watering; E_{tree} will be monitored all the time. Stem and leaf samples will be taken to measure the hydraulic recovery. Leaf area and basal stem area will be measured as well.

Data collected on xylem refilling, photosynthetic capacity, transpiration rate, and photosynthesis rate will be used to test the hardwired risk function and ABA after-effect by comparing to model outputs. **Potential pitfalls**: a hardwired risk function might not be distinguished from impacts from xylem refilling, photosynthetic capacity change, and ABA after-effect.

(3) Test optimal investments under stable environment

Two groups of six trees are grown in the greenhouse and treated with two constant watering levels. The first group is well watered and the second group is subjected to sustain moderate drought. Environmental conditions will be monitored in the greenhouse, such as temperature, light resource, atmospheric humidity, and daylight time. Predawn leaf water potential will be periodically measured to help adjust the irrigation to the two groups. Leaf life span will be monitored for the two groups to calculate the construction cost in leaves. After growth for several months, the leaf area per basal area, photosynthetic capacity, leaf mass per area and root-stem biomass will be measured.

Environmental conditions including soil moisture, root-to-shoot ratio, leaf life span, and leaf mass per area will be modeled into CMH to compute the optimal leaf investments into leaf area and photosynthetic capacity; model outputs will be compared to experimental observations to test CMH predictions on optimal investments into leaves. Measured leaf area and photosynthetic capacity will be used in CMH model under different soil moisture to quantify the price of new xylem growth in root and stem. **Potential pitfalls**: respiration in stem and root is excluded from the CMH optimization theory due to the difficulty of measurement; the respiration might cause inconsistency between model and experiments.

(4) Test leaf investment adjustment strategy

(a) Leaf photosynthetic capacity adjustment. Well-watered trees will be treated with consistently less water resource. Then predawn leaf water potential and photosynthetic capacity will be monitored every day until stable values are obtained. If leaf area changes during the long-term drought, predawn leaf water potential and photosynthetic capacity will be then monitored every day. *(b) Leaf area adjustment*. Well-watered trees are treated with consistently less water resource, and predawn leaf water potential and photosynthetic capacity will be monitored every day. Trees are treated with different drought stress length, e.g. 10, 15, 20, 25, and 30 days. After each drought stress, trees will be moved back to well-watered environment for 3 days. Leaf area change will then be measured for each tree.

Predicted results: the timing of photosynthetic capacity change during the drought will help distinguish if there is drought threshold for photosynthetic capacity shift; if photosynthetic capacity increases after leaf shedding, it suggests asynchronous leaf shedding with photosynthetic change; leaf area change under different drought length will help quantify the threshold for leaf shedding. **Potential pitfalls**: leaves might desiccate near the edge during the drought, and such behavior makes it difficult to quantify the leaf area change.

Aim 3: Apply CMH to predict forest health

The ultimate goal of this study is to apply the CMH to make reliable predictions on forest health to climatic change. Reliable predictions requires accuracy in both gas exchange via leaves and water budget in soil. Successful modelling of gas exchange relies on setting the reasonable criterion and tracking the physiological parameters change. Since physiological parameters, such as energy allocation, vary according to environmental conditions, the tracking of such parameters depends on the model ability to make water budget. Water budget includes the sources of water (i.e. effective precipitation and ground water subsidy), the sinks of water (i.e. soil evaporation and plant transpiration), and the stocks of water (i.e. soil water redistribution and water storage in plants).

Currently, existing terrestrial models employ either empirical stomatal model or WUE model for the gas exchange accompanied by soil water budget associated with the gas exchange. The disadvantages are (1) unrealistic criterion of stomatal behavior and (2) lack of interface to track physiological shifts. Therefore, it is necessary to re-link the plant gas exchange and soil water budget by adopting the CMH stomatal model and tracking the physiological shifts to climatic change.

(1) Model description on water budget

(a) Ground water subsidy: the height of water table will be a boundary condition. Above the water table (the vadose zone), water can only be supplemented by precipitation and redistribution via root and soil. Below the water table, water is always sufficient (water potential at 0) because soil hydraulic conductance is extremely high. (b) Water infiltration: water movement between adjacent soil layers is driven by a water potential difference, and the soil vulnerability curve and soil moisture release curve are defined by van Genuchten, (1980) for given soil type. (c) Root redistribution: roots will redistribute soil water among different soil layers depending on the water potential gradient and the root hydraulic conductance among layers. (d) Soil evaporation: evaporation is the water vapor diffusion driven by vapor pressure gradient between top layer soil and atmosphere; when soil gets drier, the air-water interfaces in soil particles decreases while diffusion path length increases and thus soil evaporation drops tremendously; environmental cues like wind and vegetation ratio will influence the evaporation rate as well (Eagleson, 1982). (e) Transpiration: transpiration from trees is driven by the vapor pressure deficit across the stomata; the transpiration rate will be computed from the CMH optima. Tree canopies will be divided into two layers: sun and shade layers, and leaf water potential, light, and leaf temperature will vary between layers. (f) Precipitation: here precipitation refers to effective precipitation that actually enters the soil; rain drops intercepted by leaves and water run-off are not considered as “effective precipitation”.

Environmental conditions, such as solar radiation, air temperature, atmosphere humidity, and precipitation, will be modelled into CMH in a time manner; soil temperature and soil moisture as well as plant productivity will be modeled. If plants experience long-term climatic change, optimal energy allocation at stable environment would be applied to the modeling of physiological shifts. Assuming the model solves the root zone and water budget accurately, the time course of gas exchange as well as soil moisture can be derived directly from the climate. In this way, the relationship between drought severity and plant productivity as well as forest health can be simulated. We will focus on mono-species forest types common in the intermountain west: cottonwood (*Populus fremontii*), ponderosa pine (*Pinus ponderosa*) and aspen (*Populus tremuloides*).

(2) Research plan

Build the library of stands, climates, and hydrology. (a) Stands. Theoretical stands of each species will be parameterized to represent the full range of habitat and structure. Reference stands extracted from the FIA (Forest Inventory and Analysis) data sets for each species will be included as benchmark of actual stands. (b) Climates. Theoretical climates representing the full range of aridity and temperature will be assembled along with actual climate records for the FIA stands. (c) Hydrology. Theoretical hydrology will cover full range of groundwater subsidy from zero to full subsidy. The actual hydrology for FIA stands will also be estimated for FIA stands.

Characterize drought responses at stand level. The stand-climate-hydrology combination will be simulated in a growth season to determine how the indictors of stand health, e.g. mean or median growing season hydraulic conductance loss percent (PLC) and productivity loss percent (PLA), respond to drought severity. The PLC and PLA will be assessed in relative to unlimited groundwater supply. The drought severity can be expressed as drought index DI = (effective precipitation + groundwater) / potential evapotranspiration when groundwater is unlimited. Then important aspects of stand structure (e.g. tree height, density, stand elevation, and soil depth) can be identified as important factors to forest health. I will also run simulations under current and projected higher [CO_2] to assess the potential of higher [CO_2] to mitigate drought. I will run simulations with and without energy allocation shift to examine the importance of tracking physiological shift in CMH.

Correlate the drought response to stand productivity and mortality. The FIA stands provide actual forest compositions, climate history, hydrology, and forest growth data, and can therefore be used to correlate model outputs with actual forest health and tree mortality. The simulation data within the FIA stands and FIA data will list the deficits of productivity and stomatal conductance; and the deficits can be correlated to historical tree ring width and mortality observed in FIA stands. Productivity and mortality thresholds can be deduced from the correlation between mortality and simulated physiological deficits.

Identify at-risk stands under climate change. Simulations on variety of stand-climate-hydrology combinations together with projected drought indices and [CO_2] will provide theoretical basis to characterize the threat of climate change to the three target forest types. Spatially and temporally downscaled precipitation together with estimates of hydrological impacts of winter snowpack dynamics will be used to identify any at-risk stands of the target species. **Potential pitfalls**: although FIA data set can be used to distinguish the pricing of energy allocation in root and stem, different stands might have different timing and pricing of energy allocation shifts, and the difference could cause error in the predictions.

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