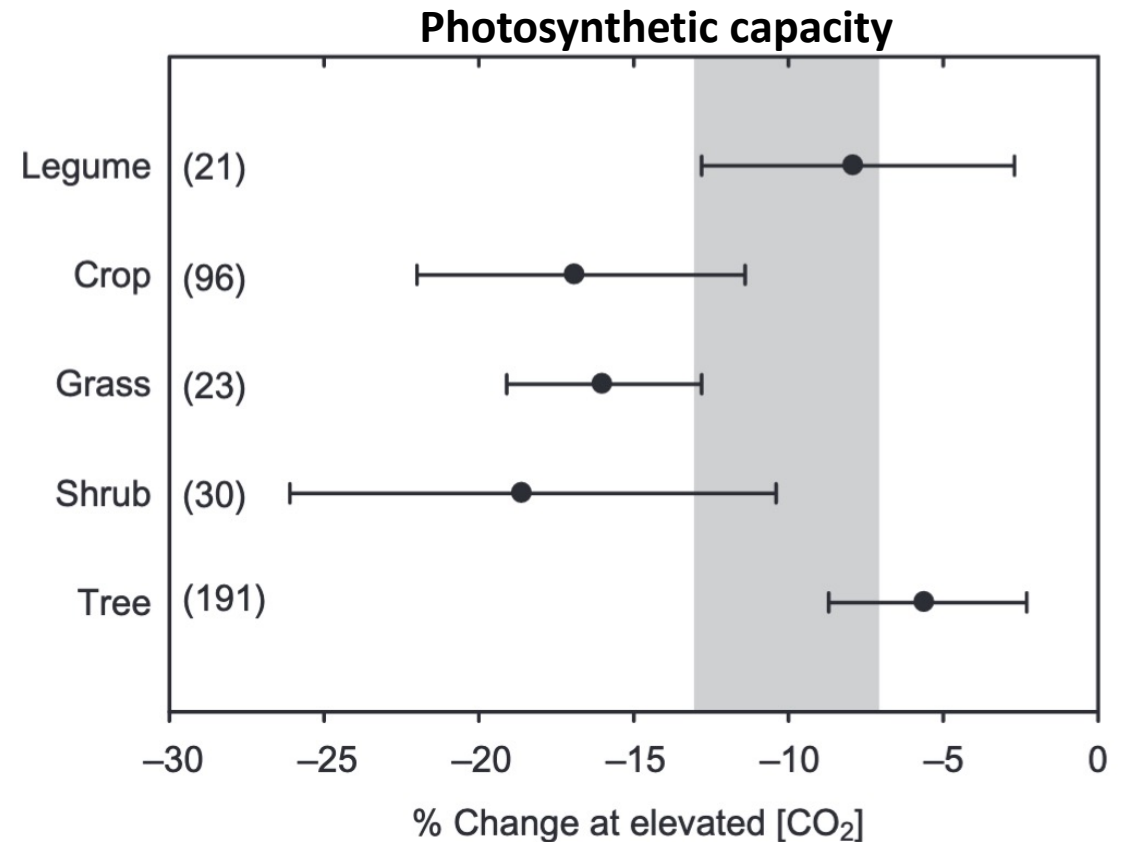
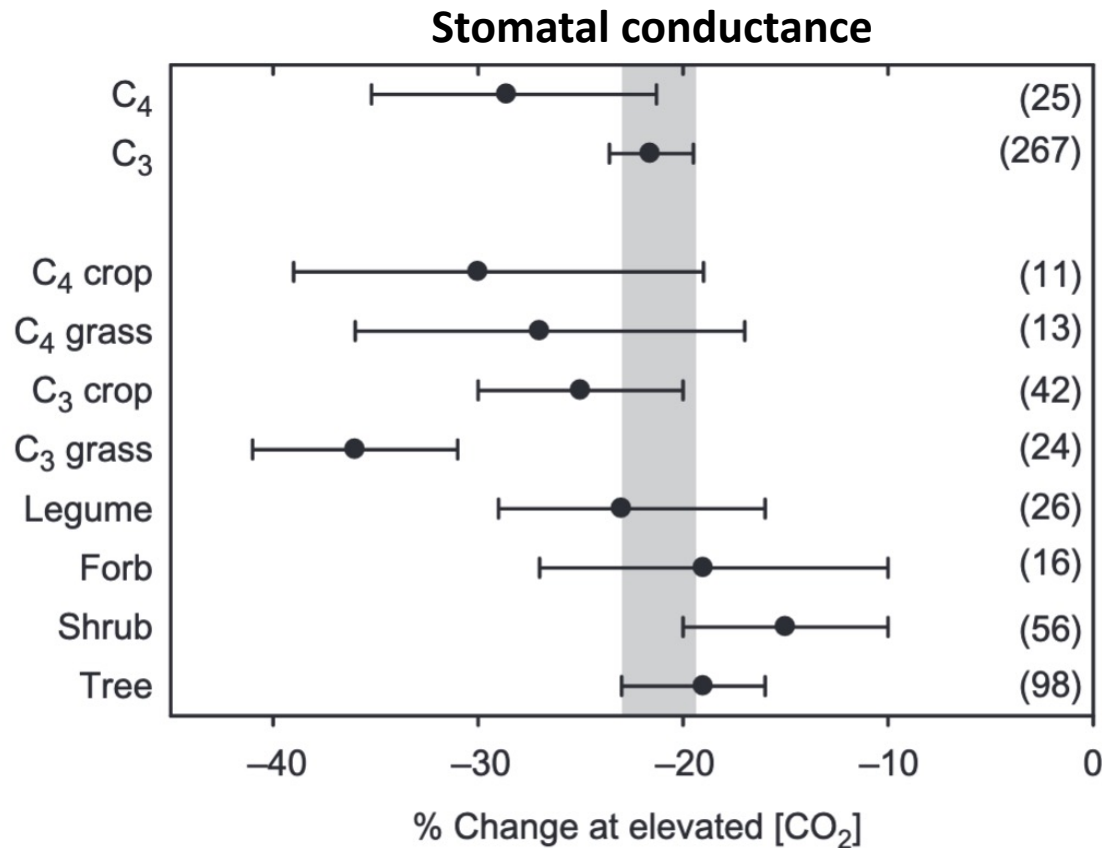


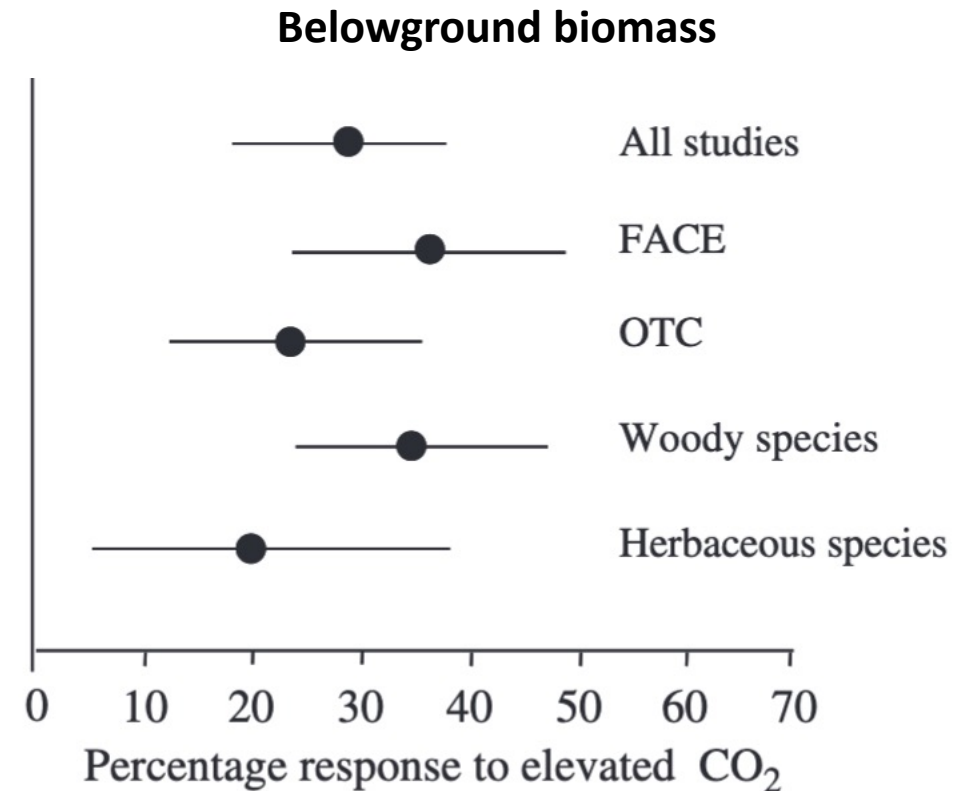
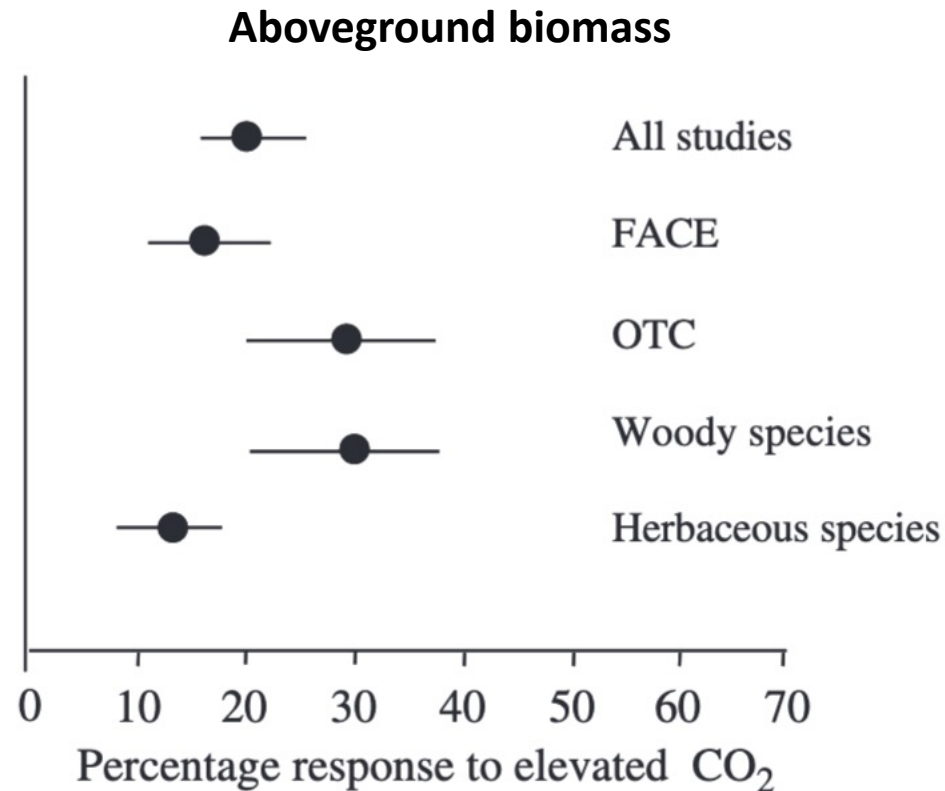
N x CO₂ x BNF growth chamber experiment

LEMONTREE Experimental Working Group – March 01, 2022

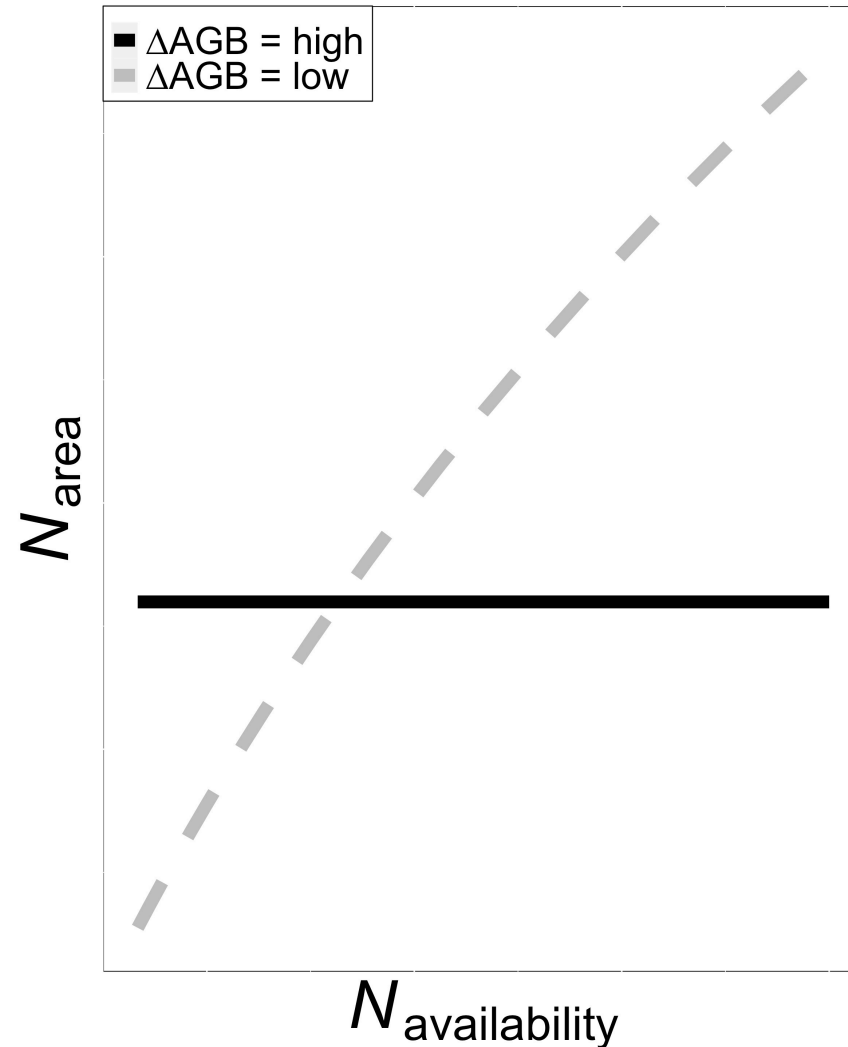
Increasing CO₂ generally decreases stomatal conductance and photosynthetic capacity...



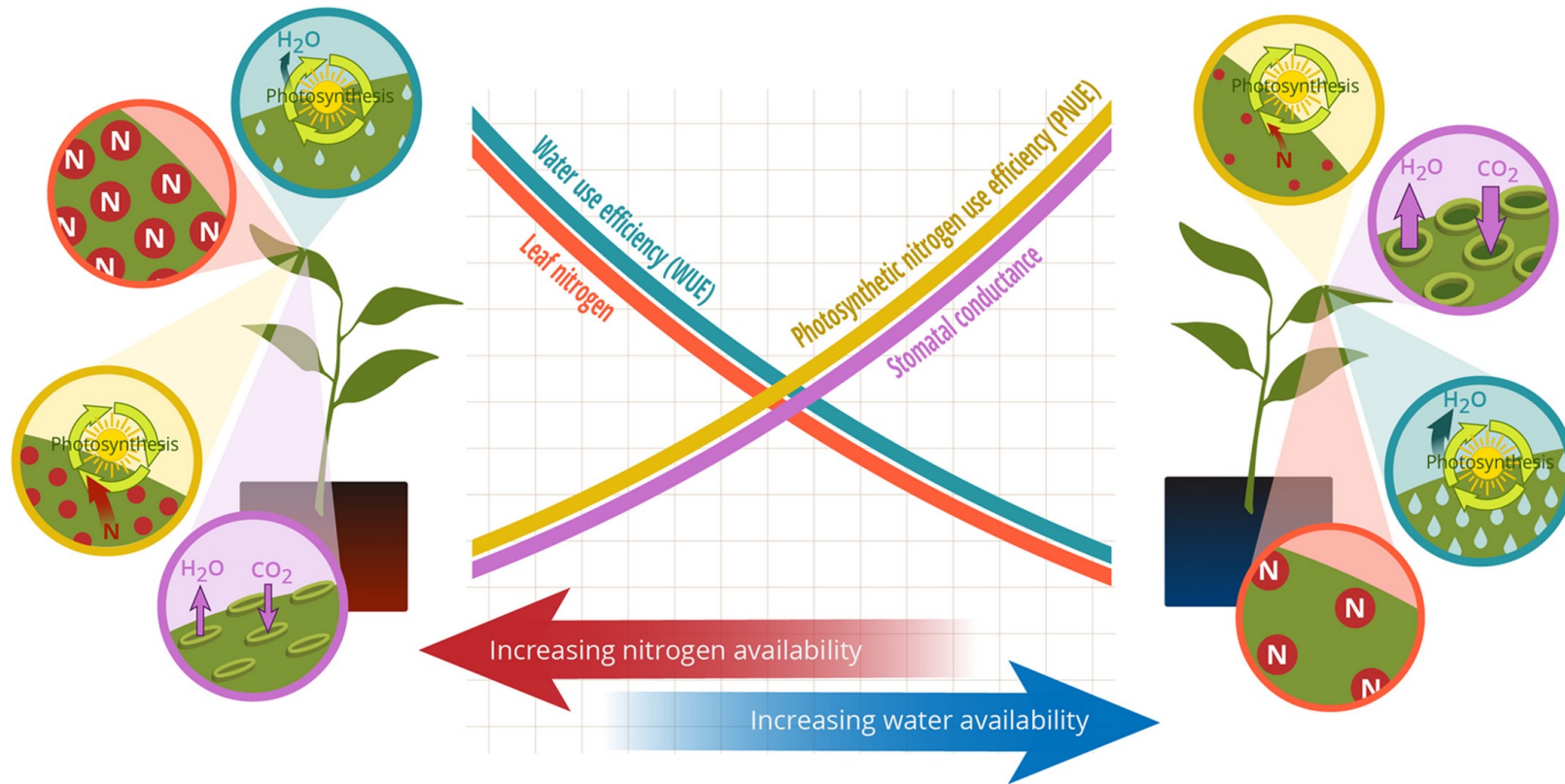
... which tends to correspond with stimulations in whole plant growth



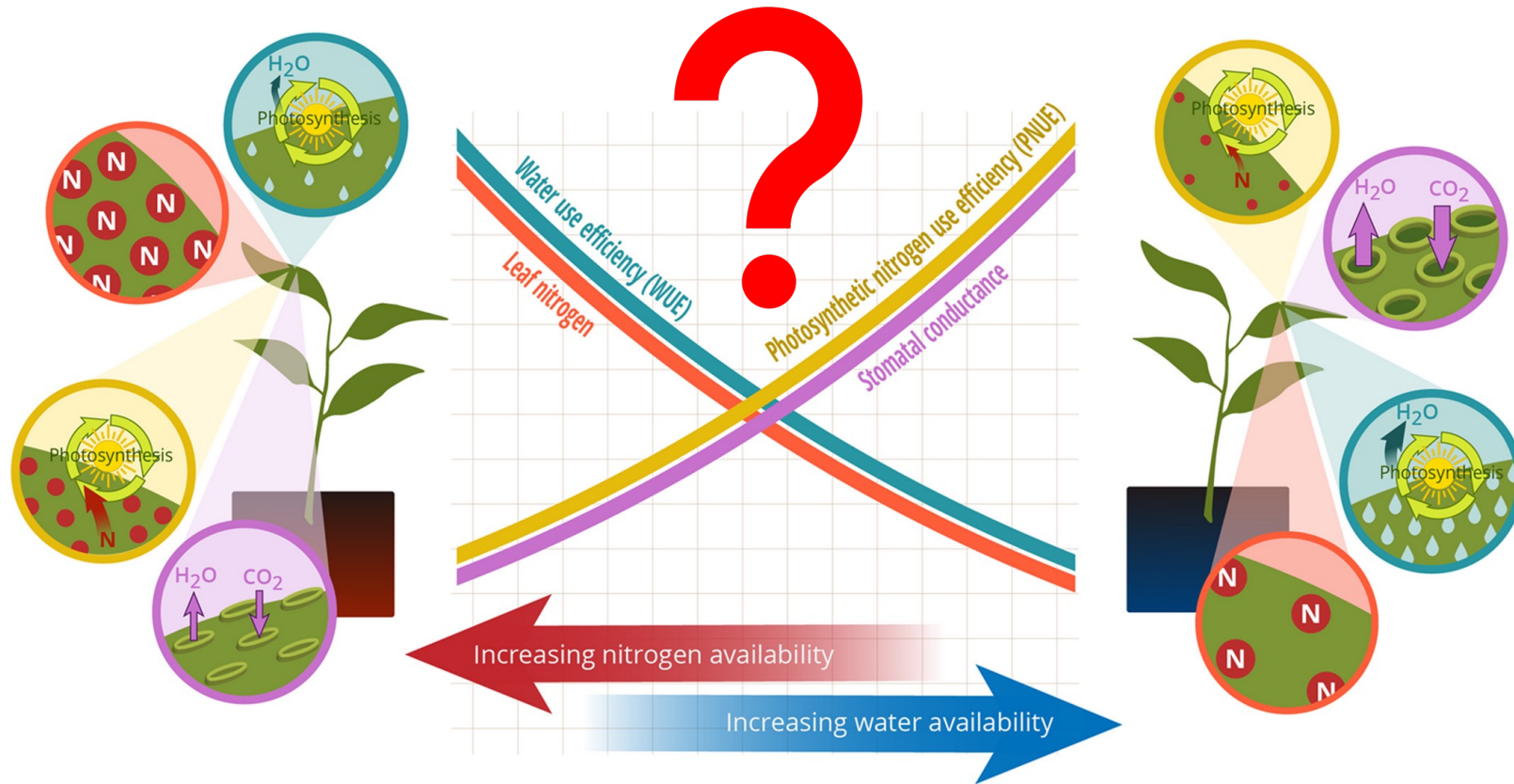
Discrepancies in leaf and whole plant responses to CO_2 may lead to tradeoffs between leaf and whole plant nutrient allocation



Maintain photosynthesis with greater **water use efficiency** at expense of **nitrogen use efficiency**, or vice versa



Maintain photosynthesis with greater **water use efficiency** at expense of **nitrogen use efficiency**, or vice versa



Key questions

How does atmospheric CO₂ modify effects of soil nutrient availability on tradeoffs between leaf nutrient allocation and whole plant growth?

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How does atmospheric CO₂ modify effects of soil nutrient availability on tradeoffs between nutrient and water use?

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How does atmospheric CO₂ modify effects of soil nutrient availability on tradeoffs between nutrient and water use?

To what extent does inoculation with nitrogen-fixing bacteria influence the two questions listed above?

Experimental setup

- Growth chamber experiment
- Individually potted soybean (*Glycine max* L.)
- Planted in unfertilized, steam sterilized potting soil
- Will grow at maximum light setting on 16:8 light: dark schedule and constant temperature (25°C) for 8-week period

Experimental setup

Nutrient acquisition strategy treatments

+ BNF

- BNF

Soil nitrogen treatments

0 ppm N

70 ppm N

140 ppm N

210 ppm N

280 ppm N

350 ppm N

Atmospheric carbon dioxide treatments

400 ppm CO₂

1000 ppm CO₂

All treatments will be combined in a full-factorial design with 6 reps per treatment combination (n = 144 total)

Plant measurements

Leaf measurements

- Leaf nitrogen allocation (N_{mass} ; SLA; N_{area})
- A_{net} , V_{cmax25} , J_{max25} , g_s , R_{d25}
- $J_{\text{max25}}:V_{\text{cmax25}}$; $R_{\text{d25}}:V_{\text{cmax25}}$; stomatal limitation
- PNUE, χ (from leaf $\delta^{13}\text{C}$), $N_{\text{area}}:g_s$, $V_{\text{cmax}}:g_s$

Whole plant measurements

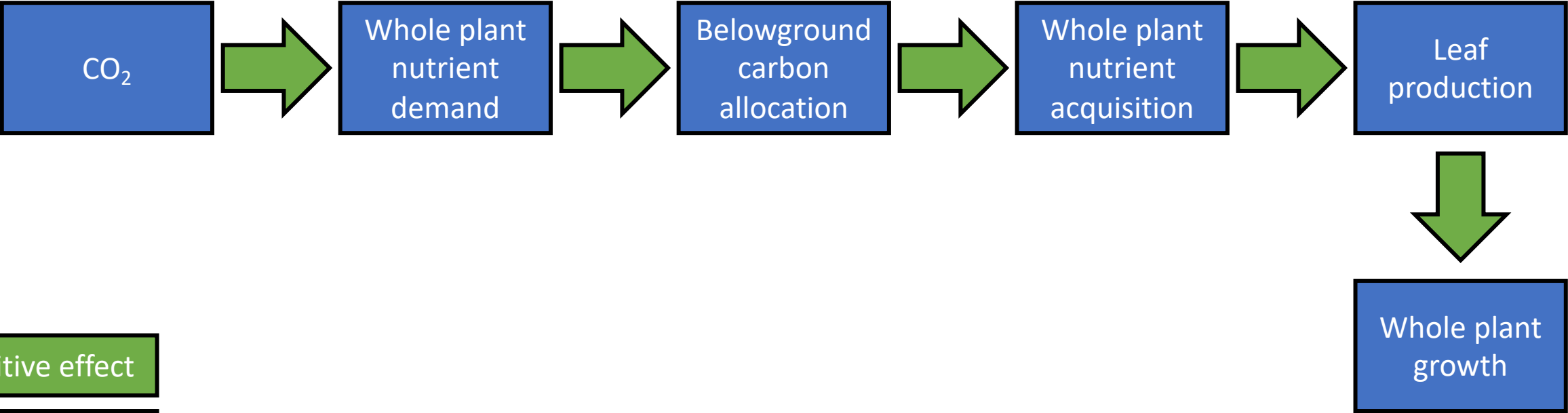
- Carbon costs to acquire nitrogen (Root carbon mass / whole plant nitrogen mass)
- Whole plant biomass
- Total leaf area
- Root nodule number, root nodule biomass

Timeline for Experiment 4

- Start experiment in March/April 2022; end in May/June 2022
- Harvest and tissue processing finished by August 2022
- Data analysis finished by September 2022
 - Could perhaps lead a working group meeting on results

Extra slides

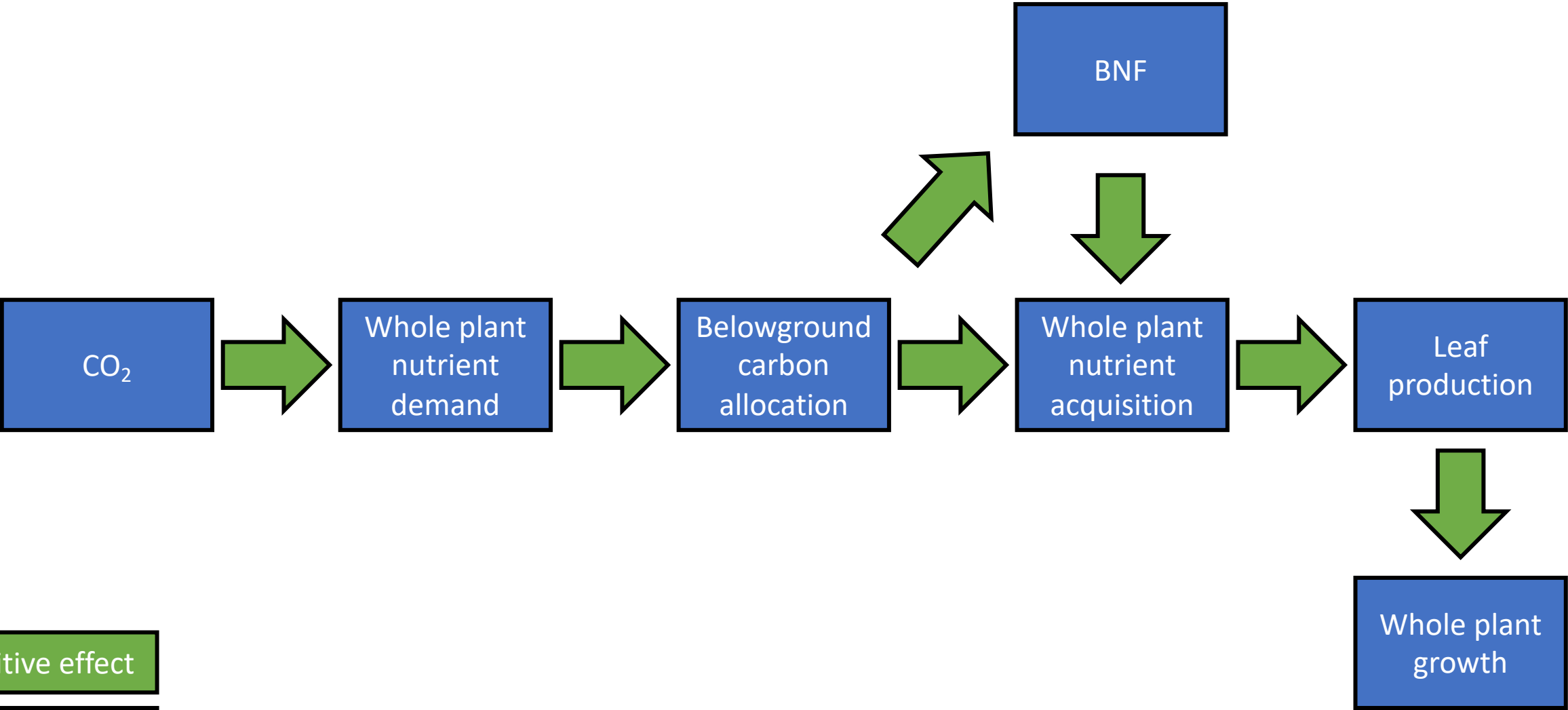
Hypothesis 1a: Increasing CO₂ should increase leaf production and whole plant growth through a stimulation in whole plant nutrient demand



Positive effect

Negative effect

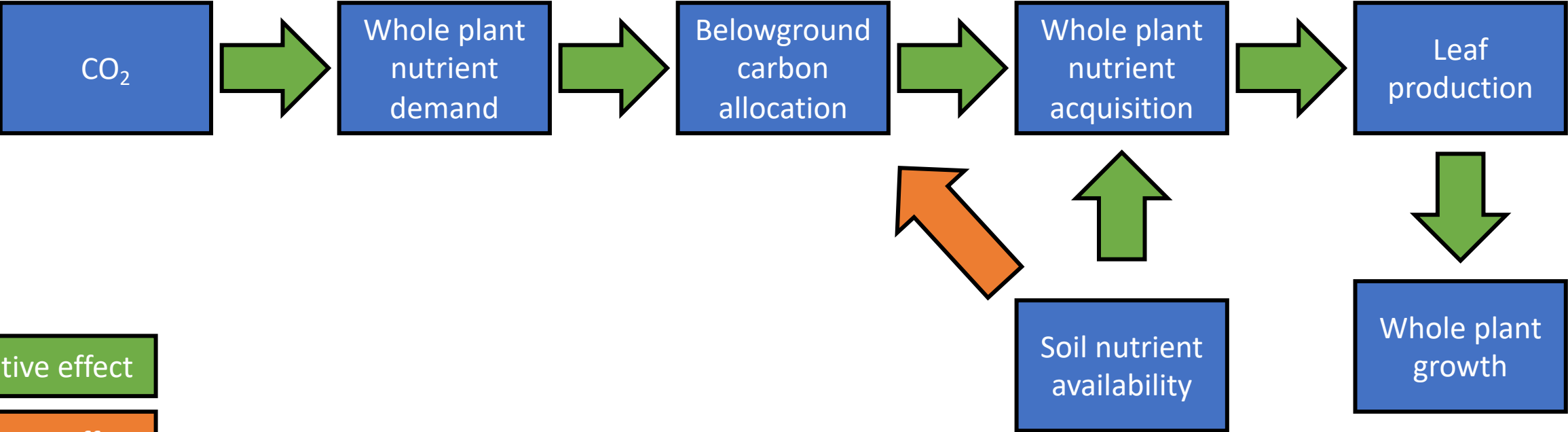
Hypothesis 1a: Increasing CO₂ should increase leaf production and whole plant growth through a stimulation in whole plant nutrient demand



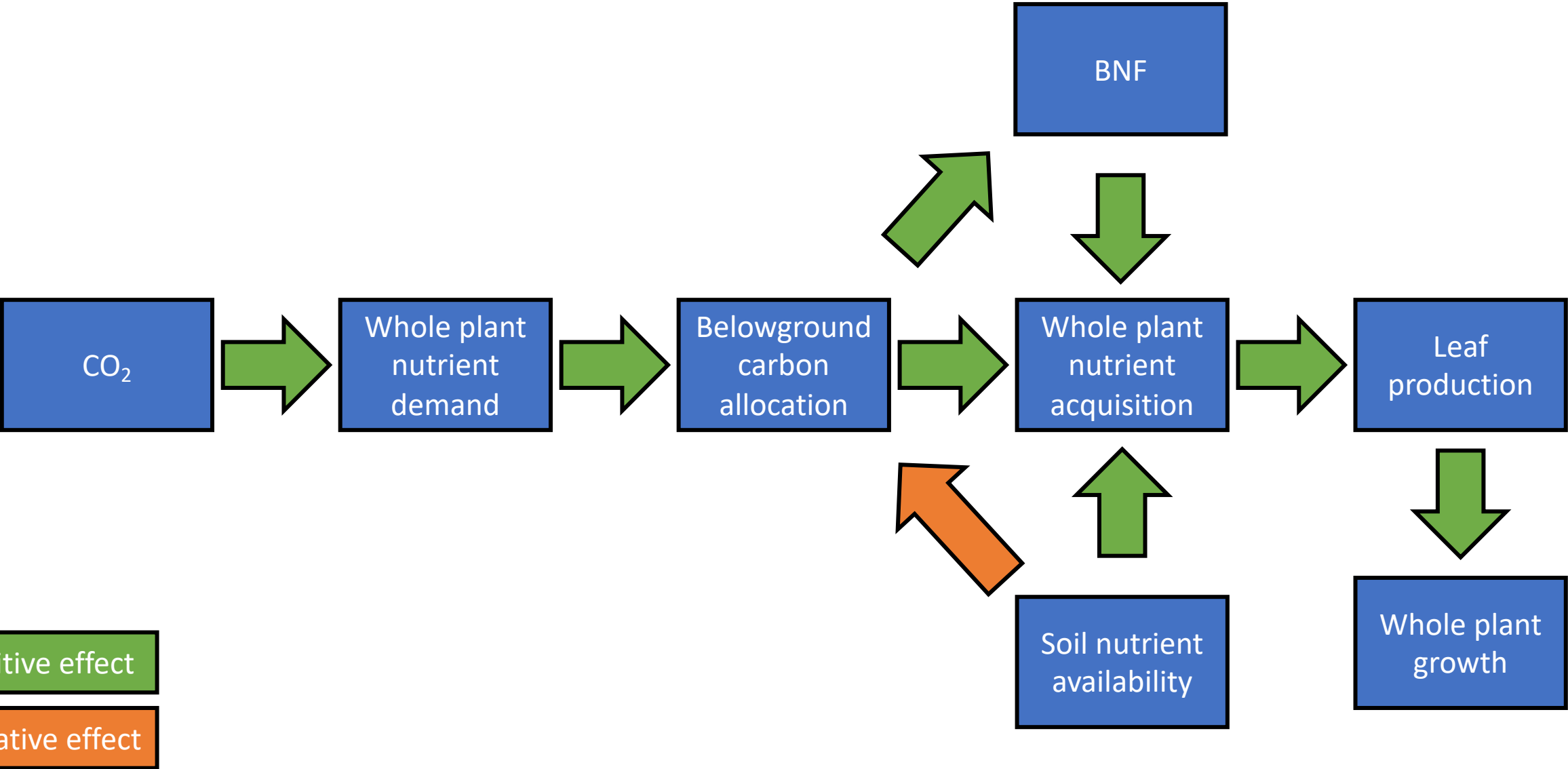
Positive effect

Negative effect

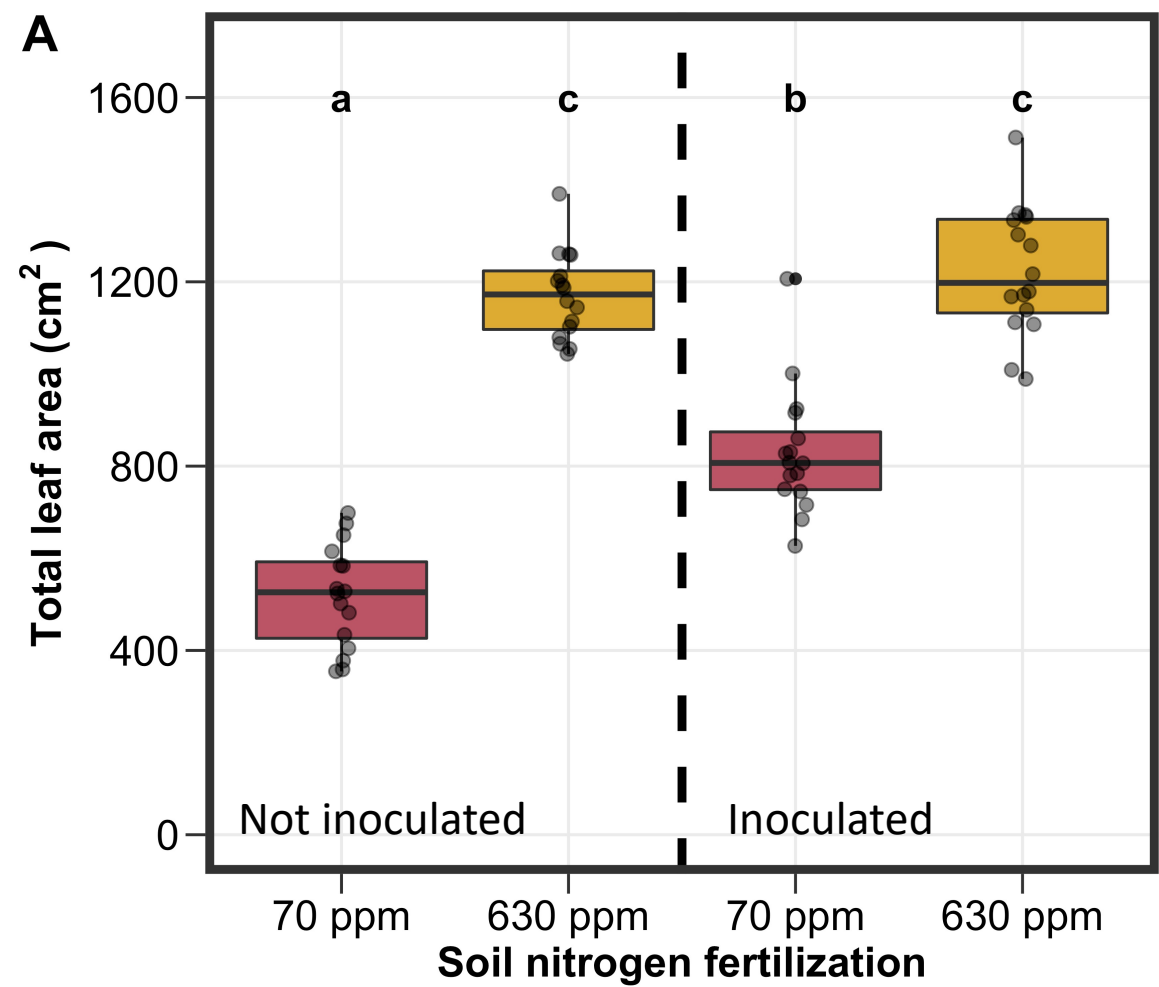
Hypothesis 1b: Increased soil nutrient supply should increase the positive effect of eCO₂ on leaf production and whole plant growth



Hypothesis 1b: Increased soil nutrient supply should increase the positive effect of eCO₂ on leaf production and whole plant growth, **but will depend on whether individuals associate with nitrogen-fixing bacteria**

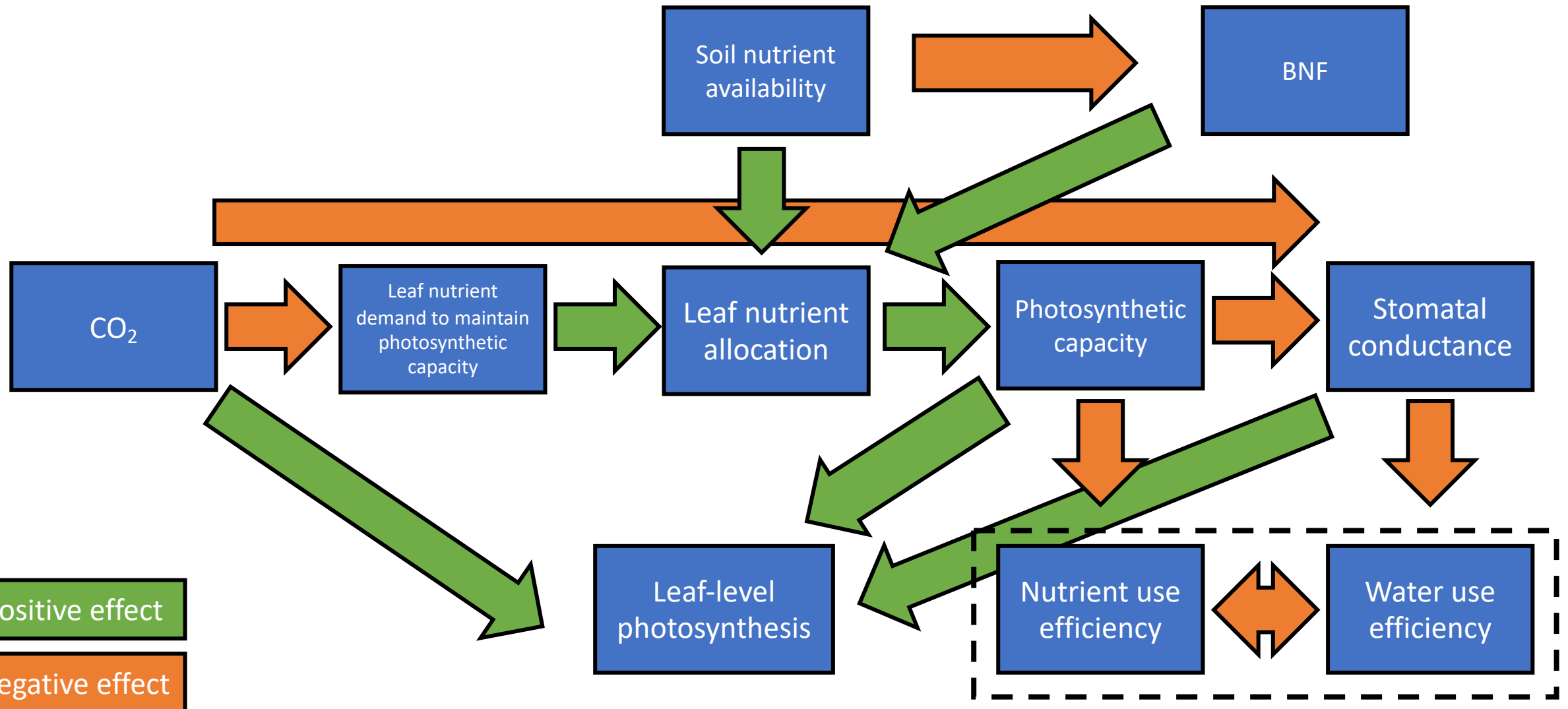


Hypothesis 1b: Increased soil nutrient supply should increase the positive effect of eCO₂ on leaf production and whole plant growth, **but will depend on whether individuals associate with nitrogen-fixing bacteria**

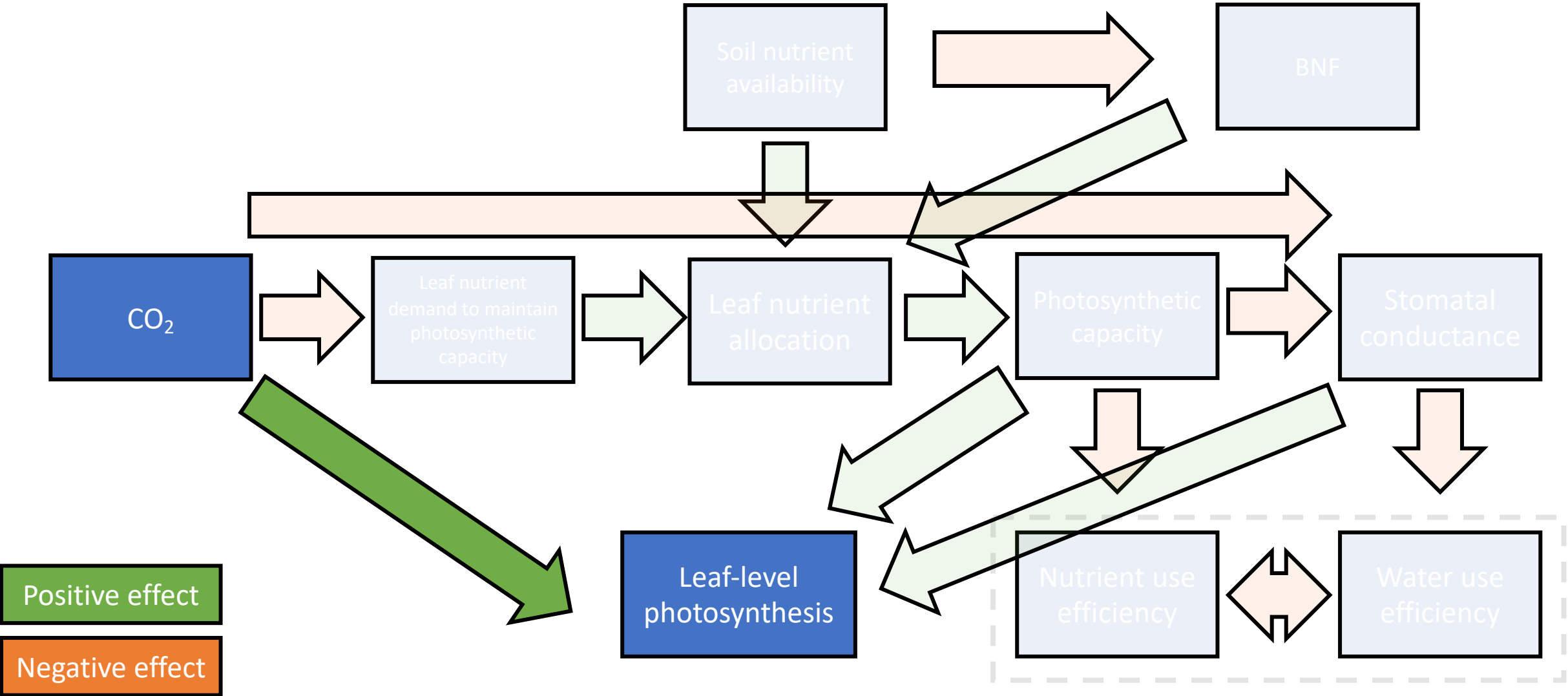


Inoculation with BNF should increase total leaf area and growth under low soil nutrients, but have similar total leaf area and growth under high soil nutrients

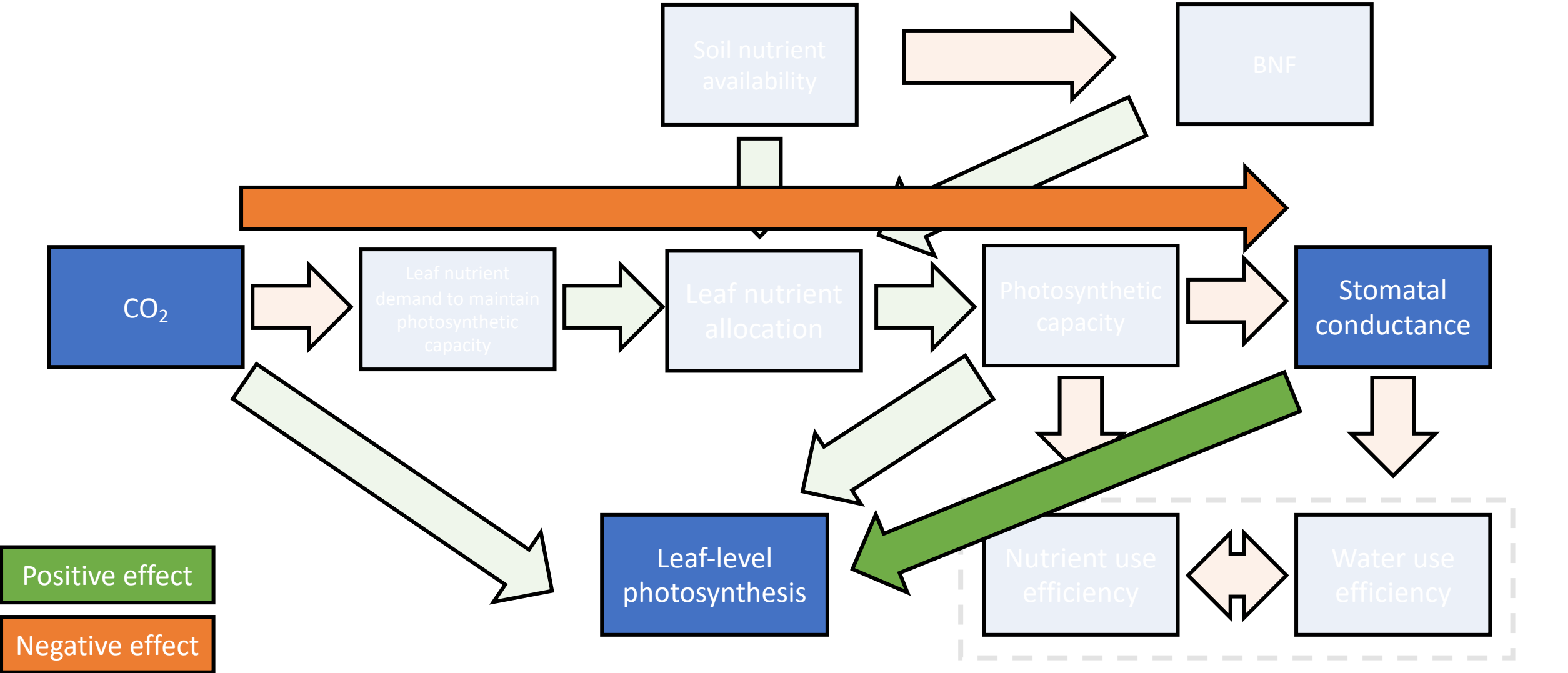
Hypothesis 2



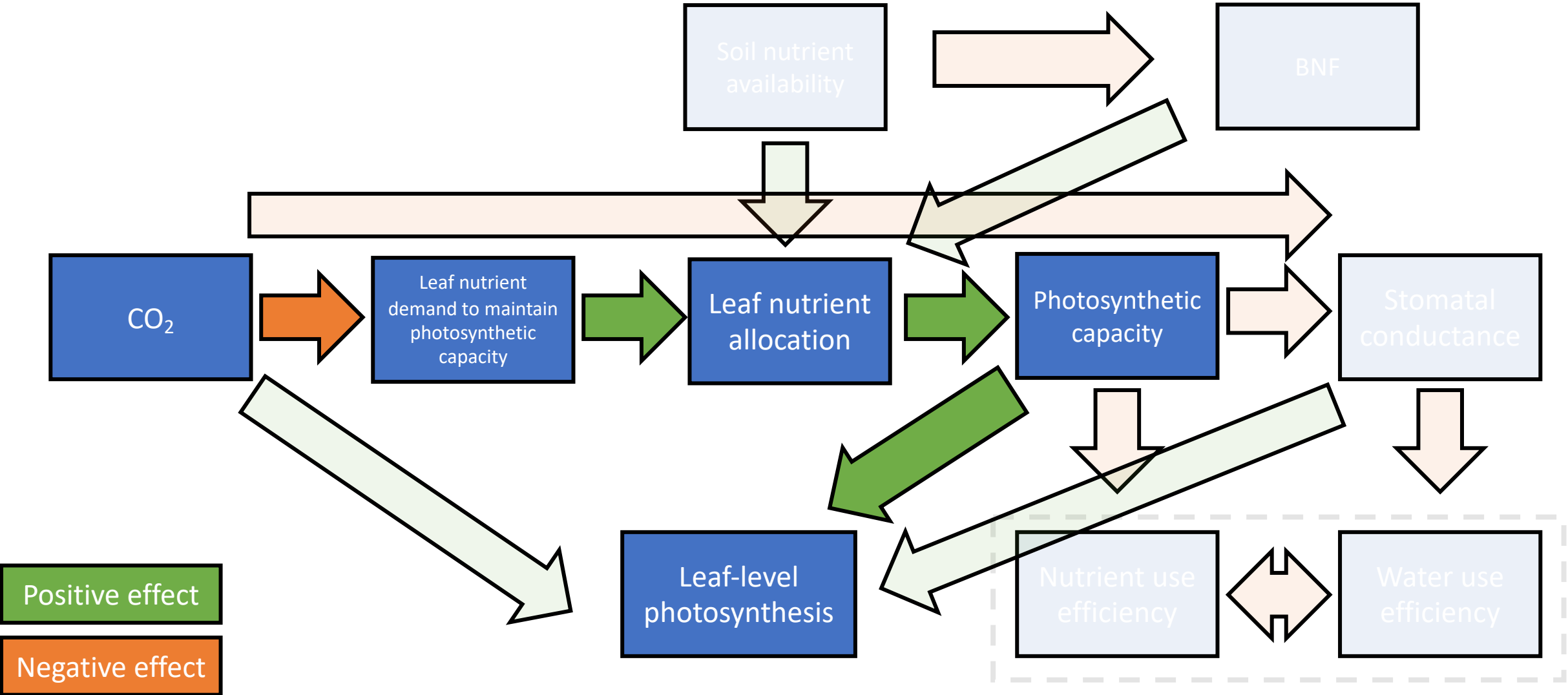
Hypothesis 2a: Increasing CO₂ will have a direct positive effect on leaf-level photosynthesis due to increased substrate needed to drive photosynthesis forward



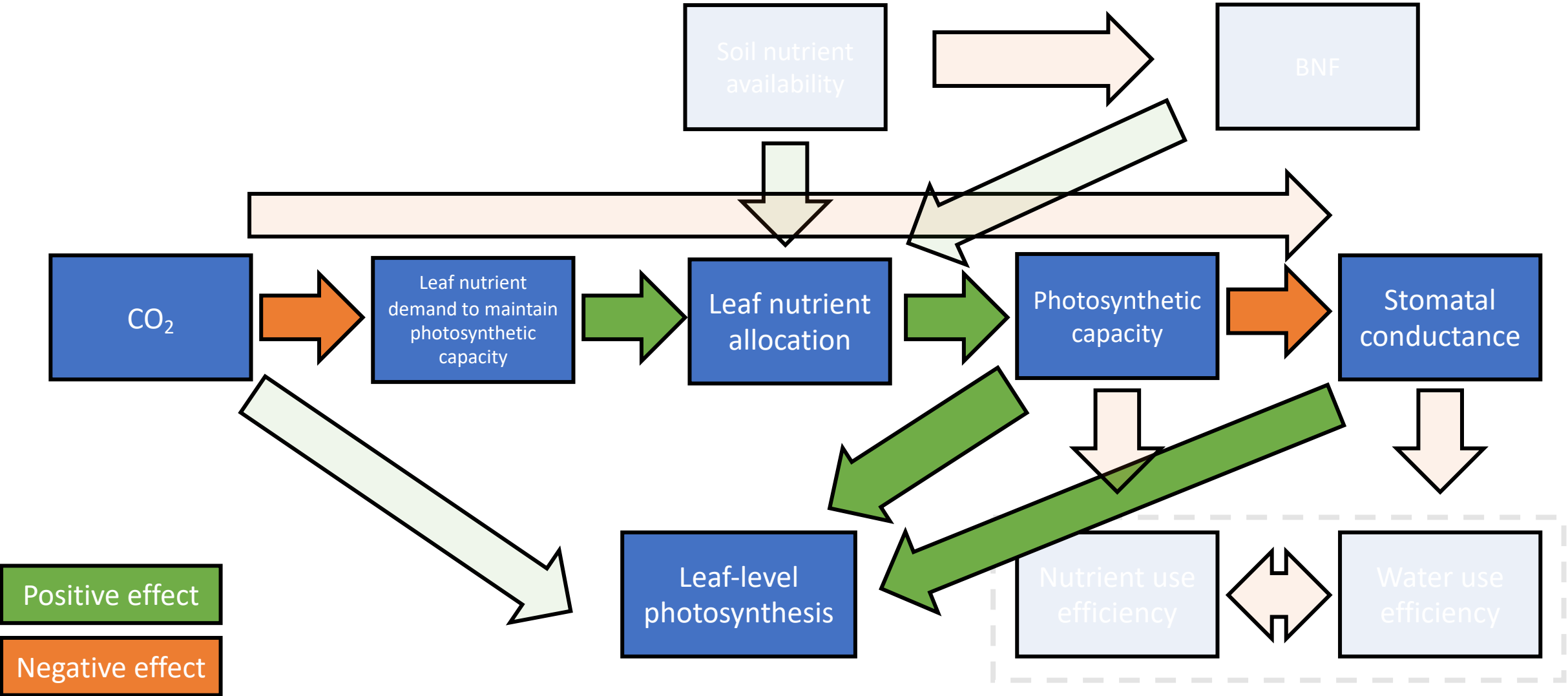
Hypothesis 2b: Increasing CO₂ will decrease leaf-level photosynthesis as a function of reduced stomatal conductance



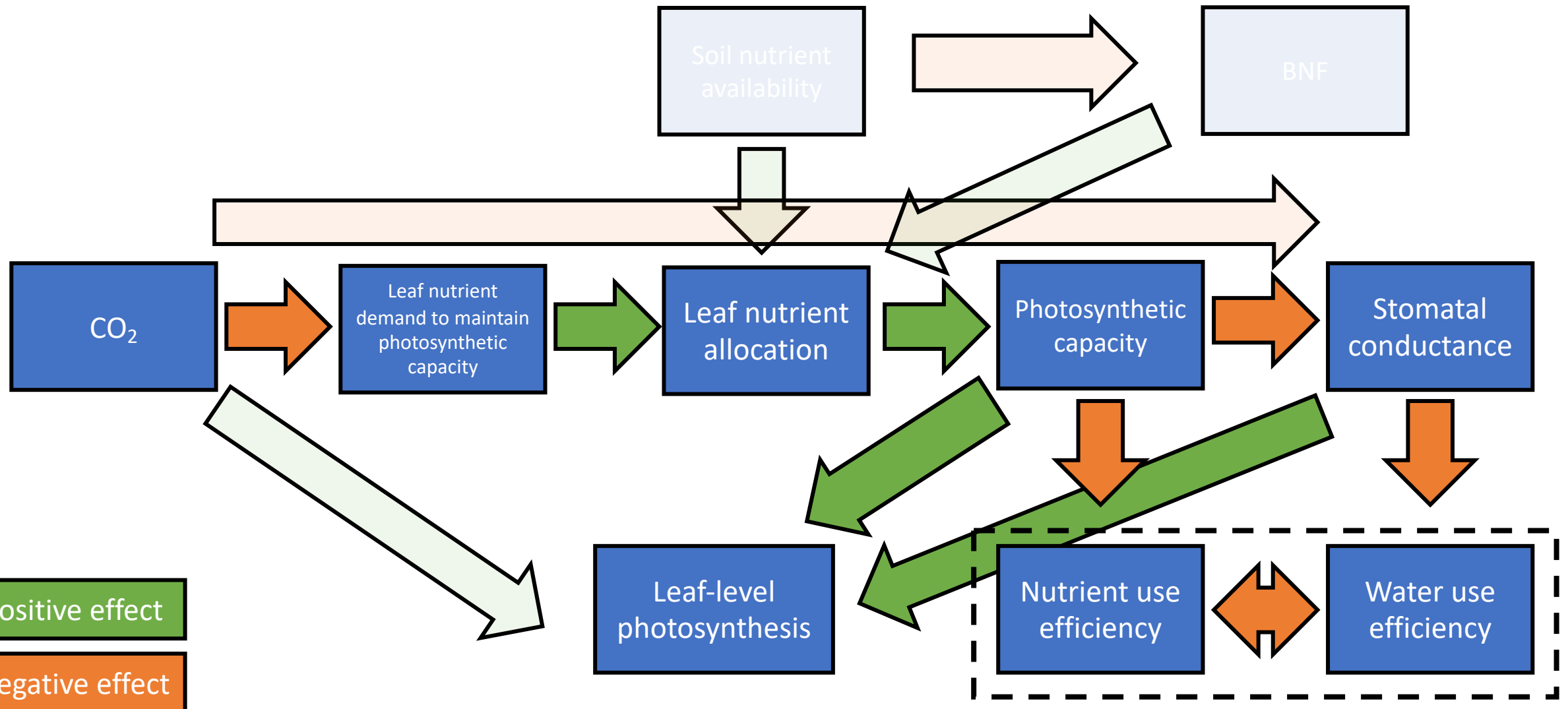
Hypothesis 2c: Increasing CO₂ will decrease leaf-level photosynthesis as a function of reduced maintenance costs for photosynthetic capacity



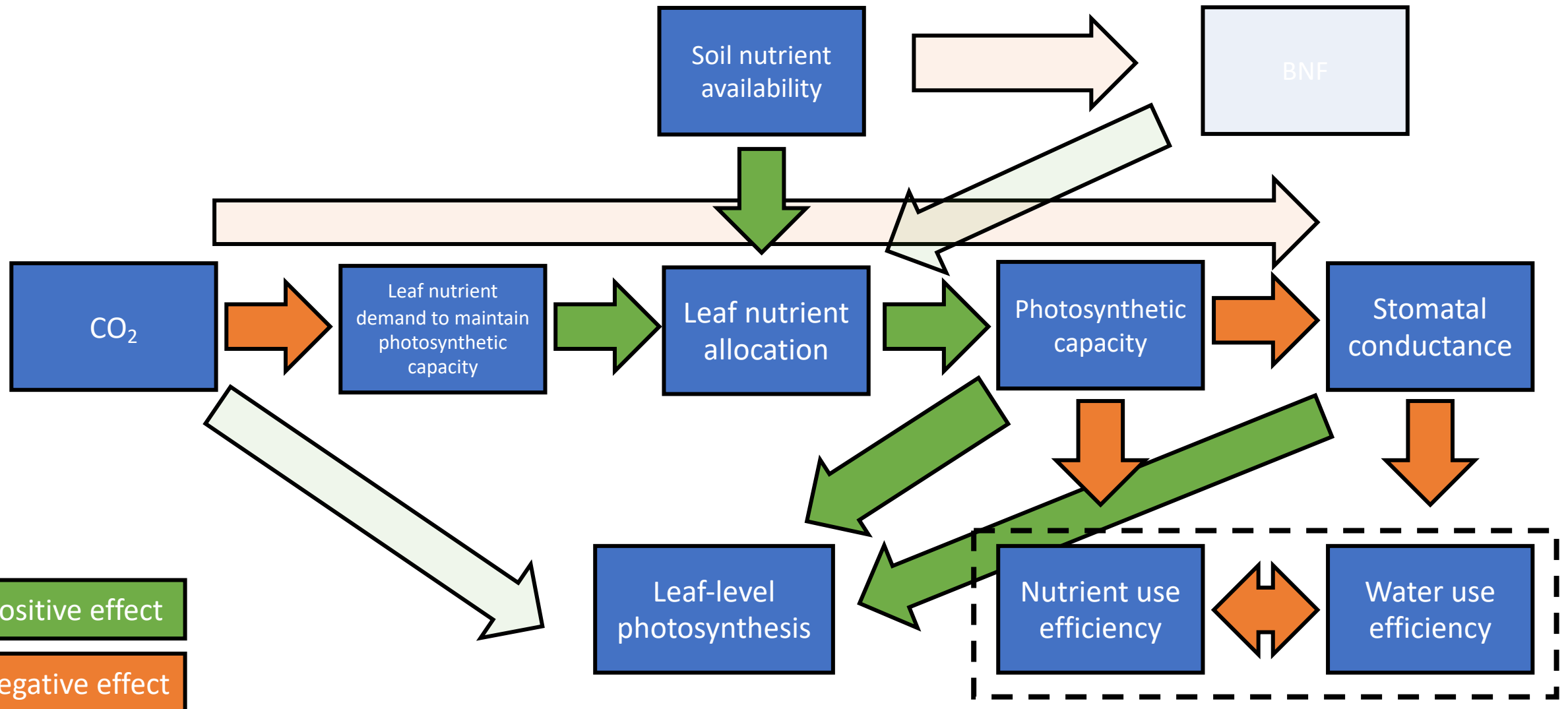
Hypothesis 2c: Increasing CO₂ will decrease leaf-level photosynthesis as a function of reduced maintenance costs for photosynthetic capacity



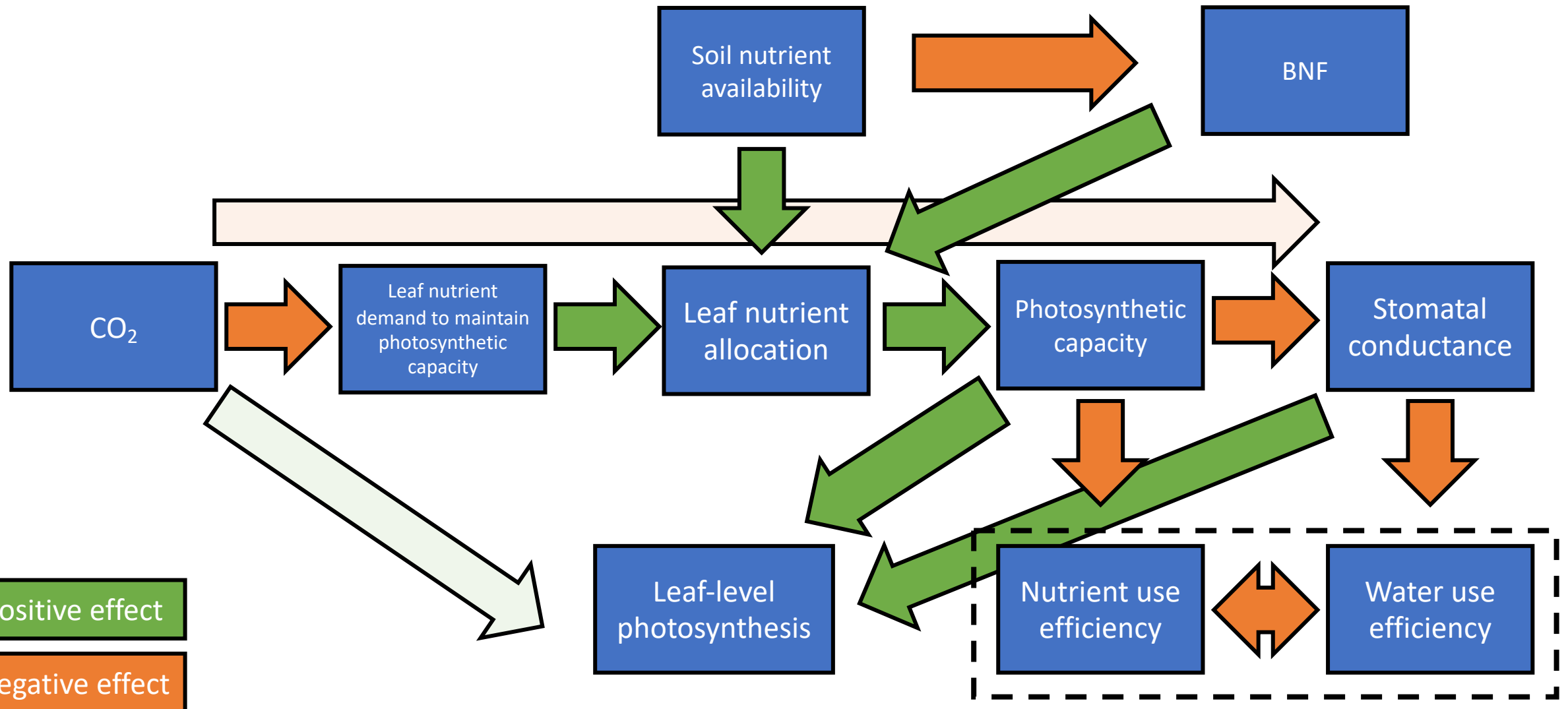
Hypothesis 2c: Increasing CO₂ will decrease leaf-level photosynthesis as a function of reduced maintenance costs for photosynthetic capacity



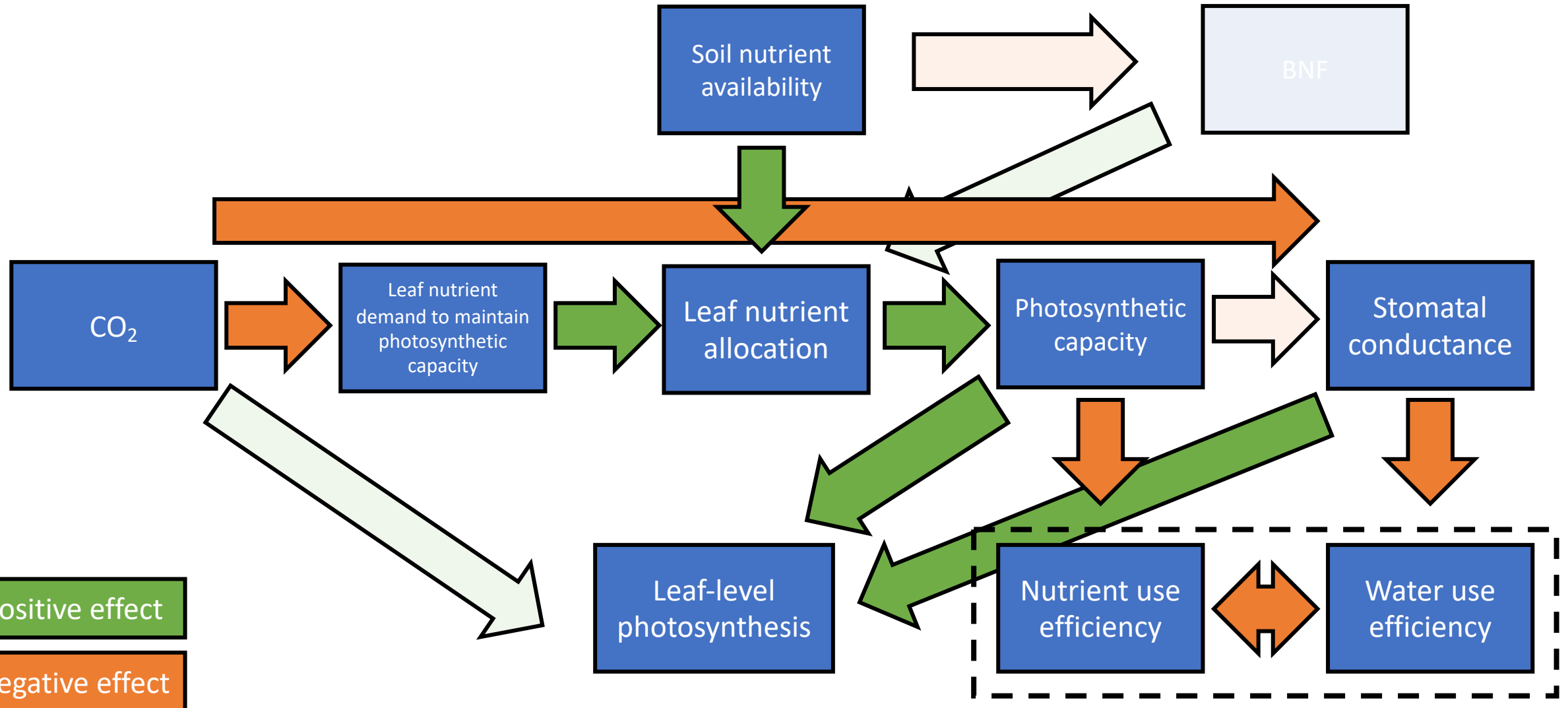
Hypothesis 2c: Increasing CO₂ will decrease leaf-level photosynthesis as a function of reduced maintenance costs for photosynthetic capacity



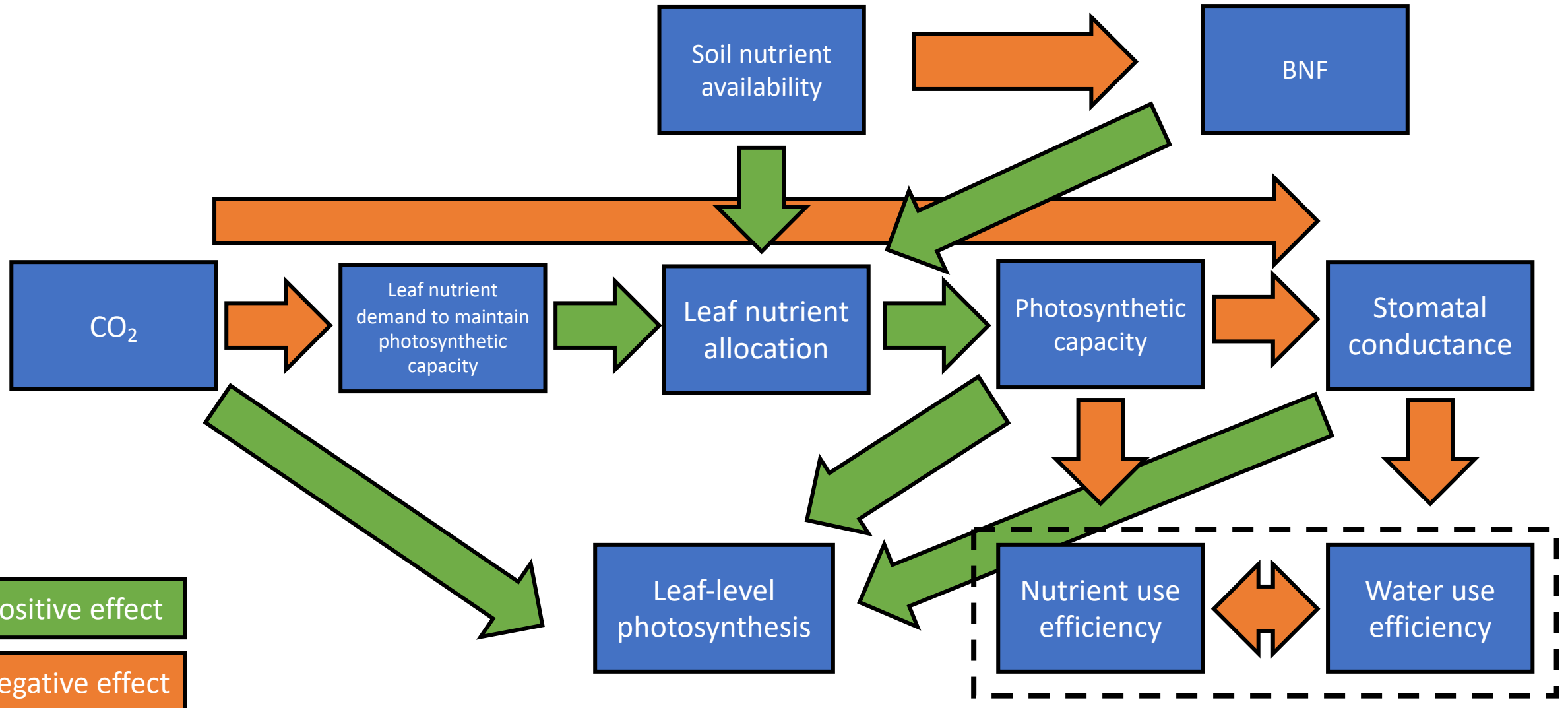
Hypothesis 2c: Increasing CO₂ will decrease leaf-level photosynthesis as a function of reduced maintenance costs for photosynthetic capacity



Hypothesis 2d: Increasing CO₂ will decrease leaf-level photosynthesis as a function of reduced maintenance costs for photosynthetic capacity and reduced stomatal conductance



Hypothesis 2



Timeline for Experiment 4

- Start experiment in March/April 2022 using growth chambers in ESB I
- Only one growth chamber available
 - Two separate trial runs for each CO₂ treatment (2 months each)
 - Temperature, relative humidity, and PAR sensors set up through both trials
- Harvest and tissue processing should take another 2 months
- Data analysis, manuscript draft finished by end of calendar year?

Products from Experiment 4

- 1-2 publications
 - Structural carbon costs to acquire nitrogen responses to N fixation, N fertilization, and CO₂
 - Leaf and whole plant nitrogen allocation responses to N fixation, N fertilization, and CO₂
- A few conferences in winter 2022 (AGU?) and summer 2023 (BSA/ESA)