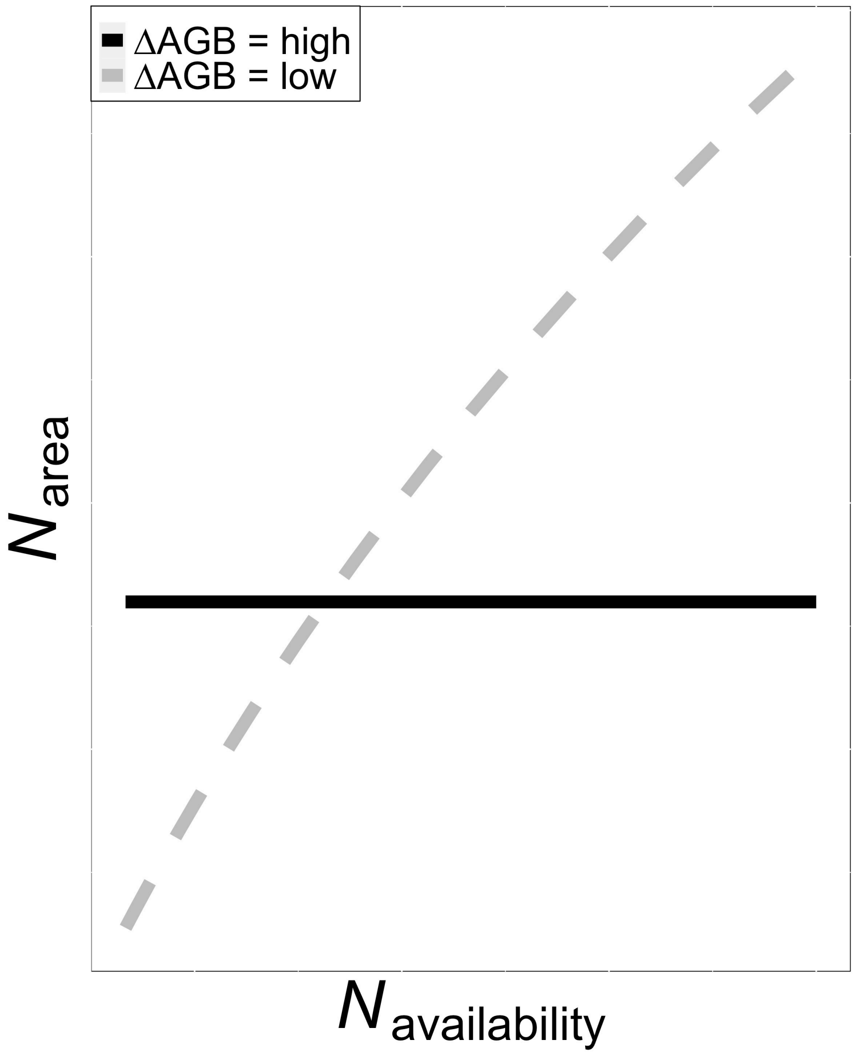
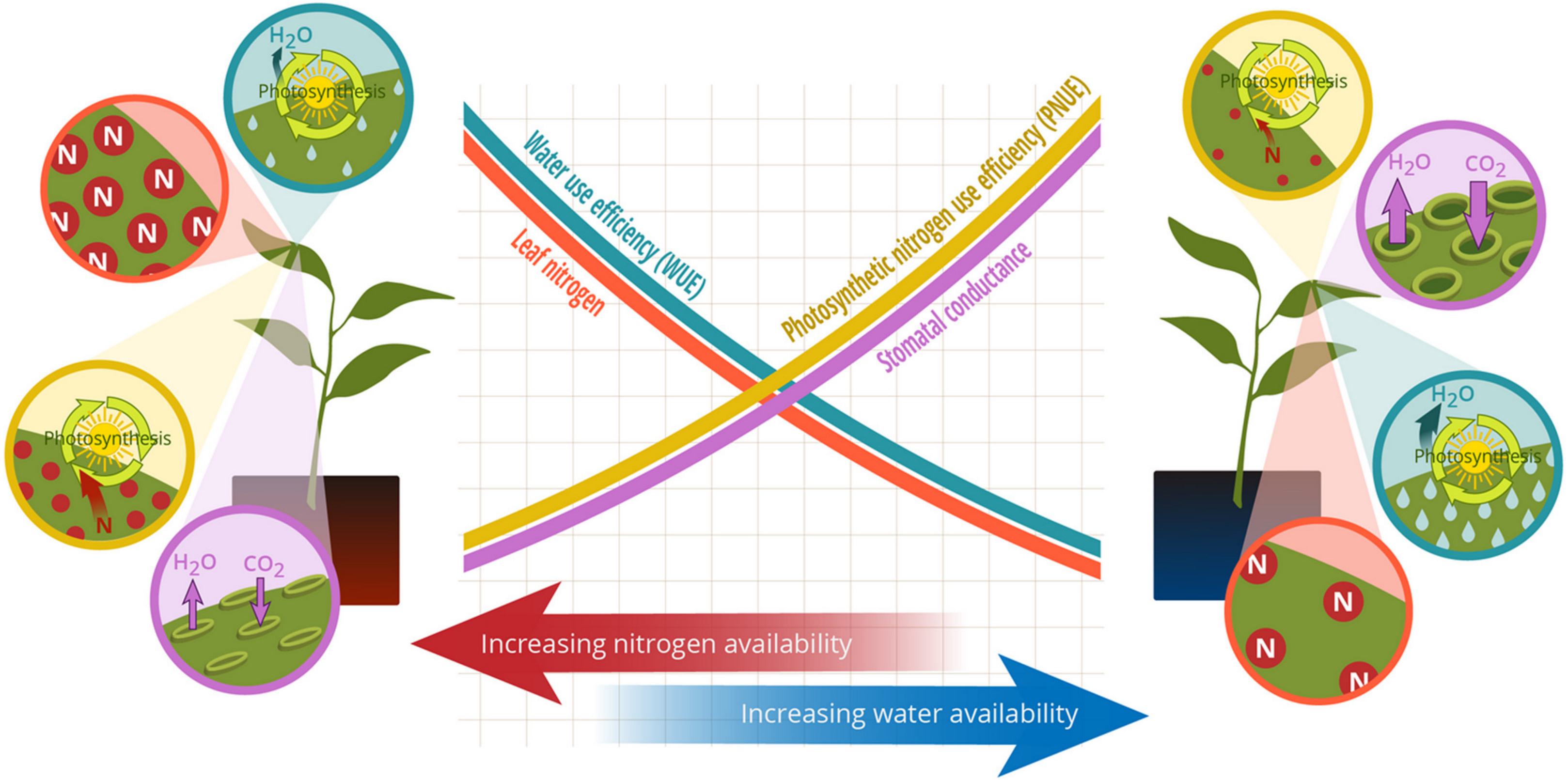
Experimental Chapter 4: N x CO_2 x BNF growth chamber experiment

Option #1: Invest extra nitrogen toward whole plant growth at expense of leaf nitrogen



Smith et al. (in prep)

Option #2: Maintain photosynthesis with greater water use efficiency at expense of nitrogen use efficiency



Bialic-Murphy *et al.* (2021) *Ecol. Letters*

Key questions

How does atmospheric CO_2 modify effects of soil nutrient availability on tradeoffs between leaf nutrient allocation and whole plant growth?

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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*)
- 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
- 210 ppm N
- 630 ppm N

Atmospheric carbon dioxide treatments

- 400 ppm CO_2
- 1000 ppm CO_2

All treatments will be combined in a full-factorial design with 10 reps per treatment combination (n = 160 total)

Plant measurements

Leaf measurements

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

Whole plant measurements

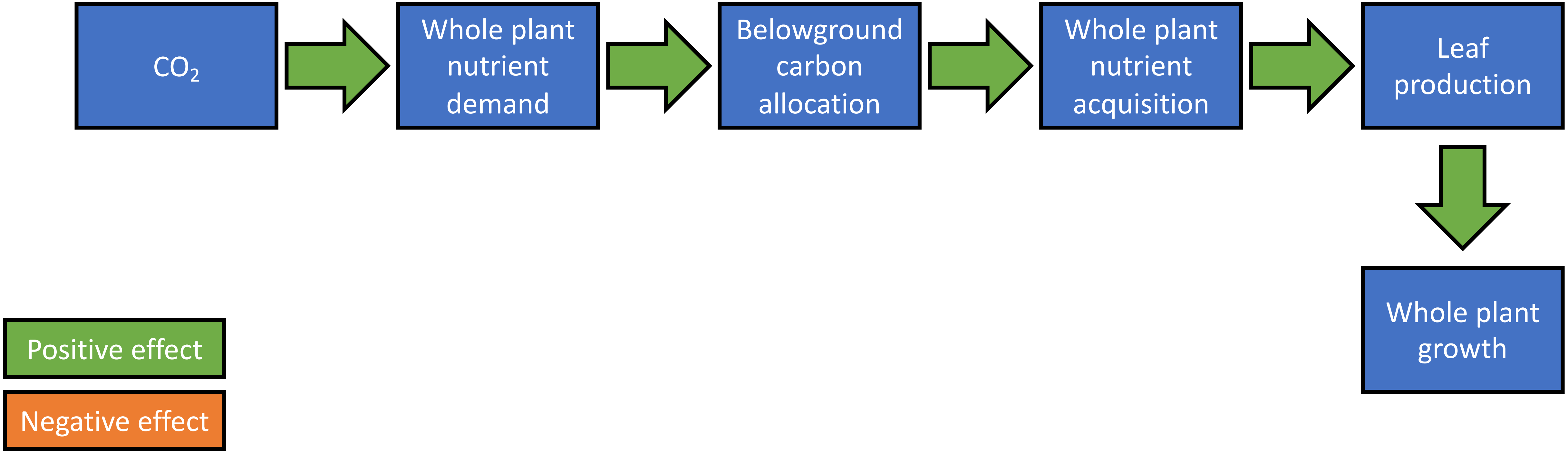
- Carbon costs to acquire nitrogen (Root carbon mass / whole plant nitrogen mass)
- Whole plant biomass
- Total leaf area
- Root nodule number, root nodule biomass

Timeline for Experiment 4

- Start experiment in March/April 2022 using growth chambers in ESB I
- Only one growth chamber available
  - Two separate trial runs for each CO_2 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?

Extra slides for hypotheses

Hypothesis 1a: Increasing CO_2 should increase leaf production and whole plant growth through a stimulation in whole plant nutrient demand



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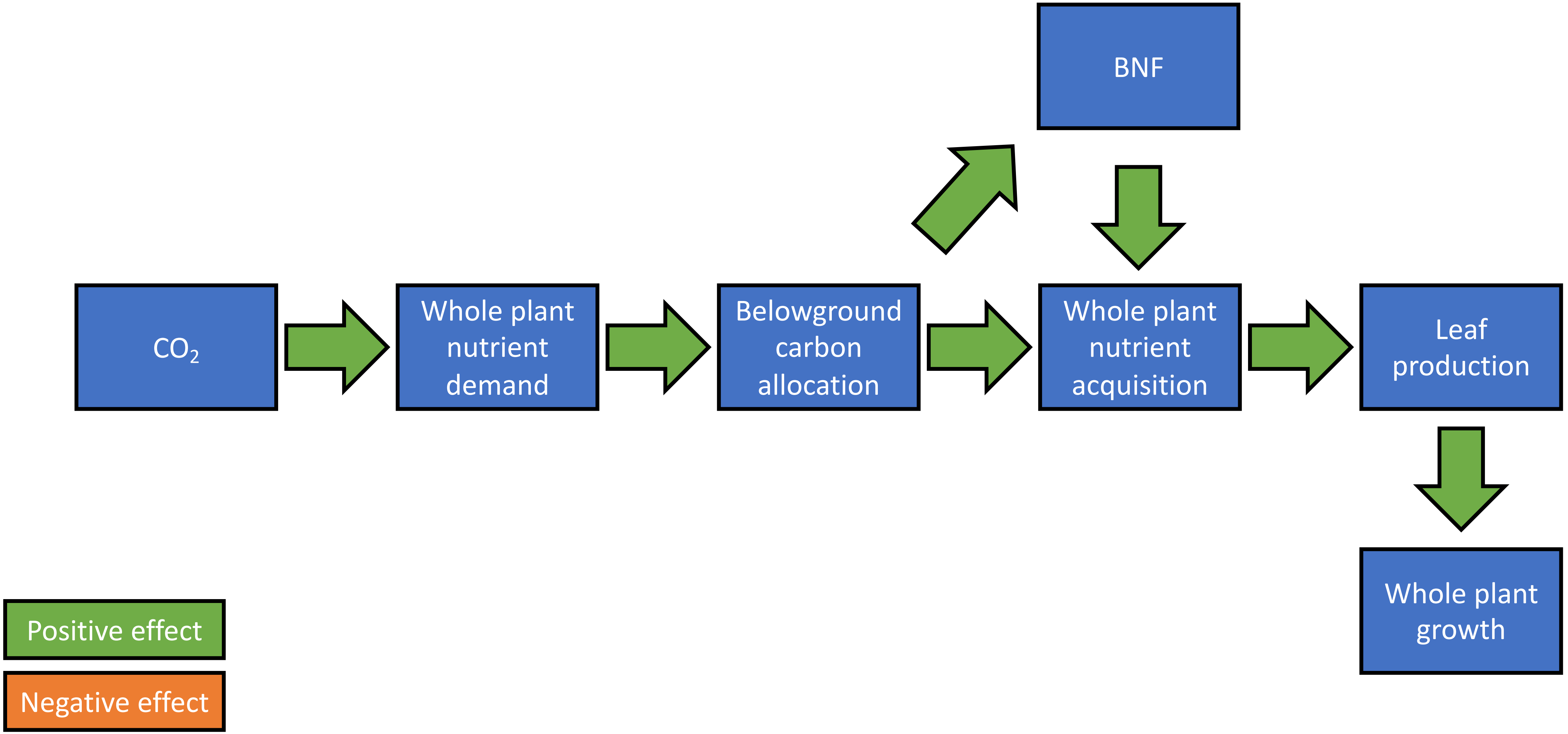
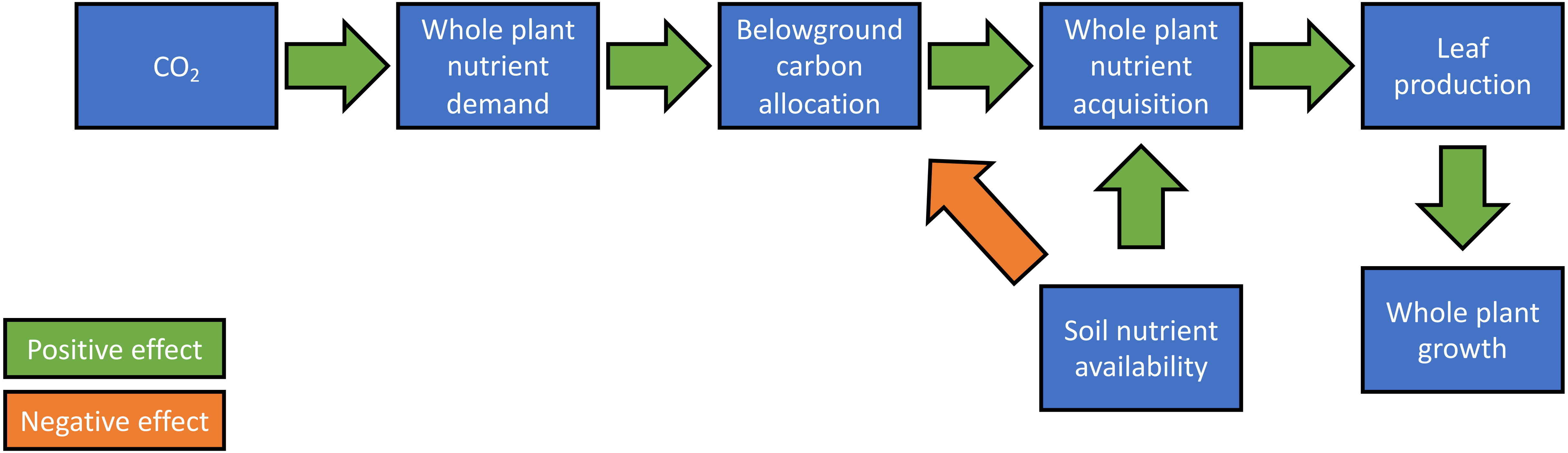
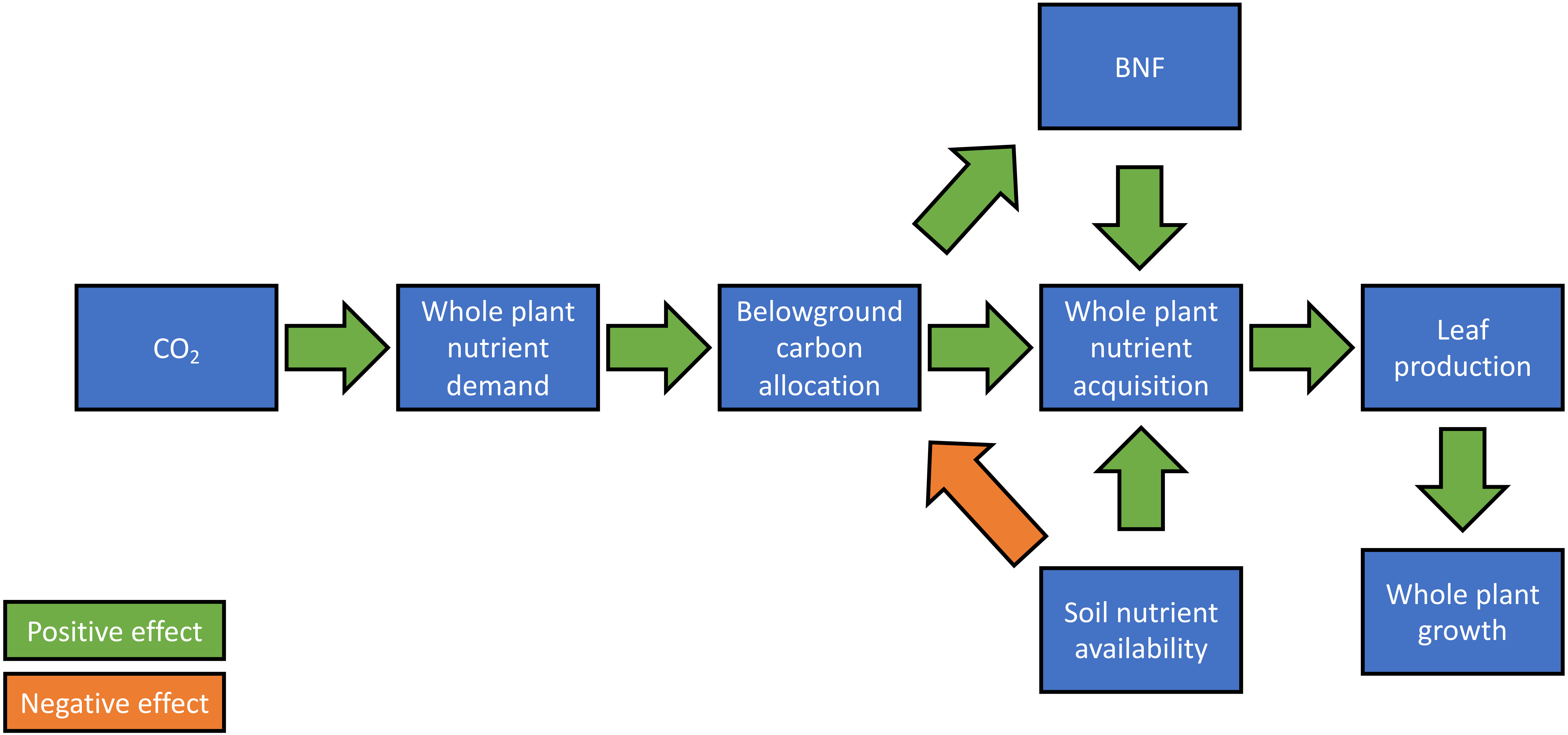


Hypothesis 1b: Increased soil nutrient supply should increase the positive effect of eCO_2 on leaf production and whole plant growth



Hypothesis 1b: Increased soil nutrient supply should increase the positive effect of eCO_2 on leaf production and whole plant growth, **but will depend on whether individuals associate with nitrogen-fixing bacteria**



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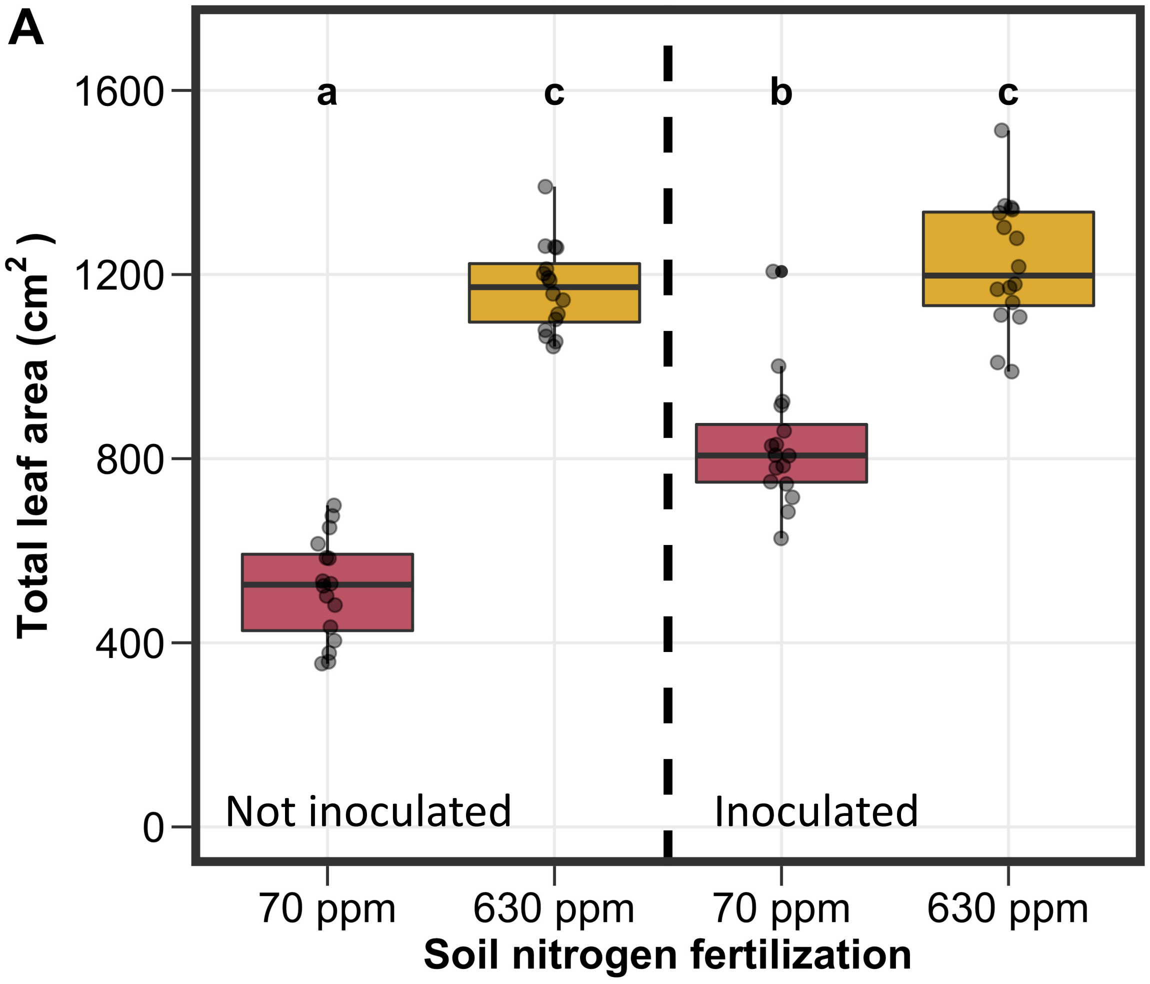
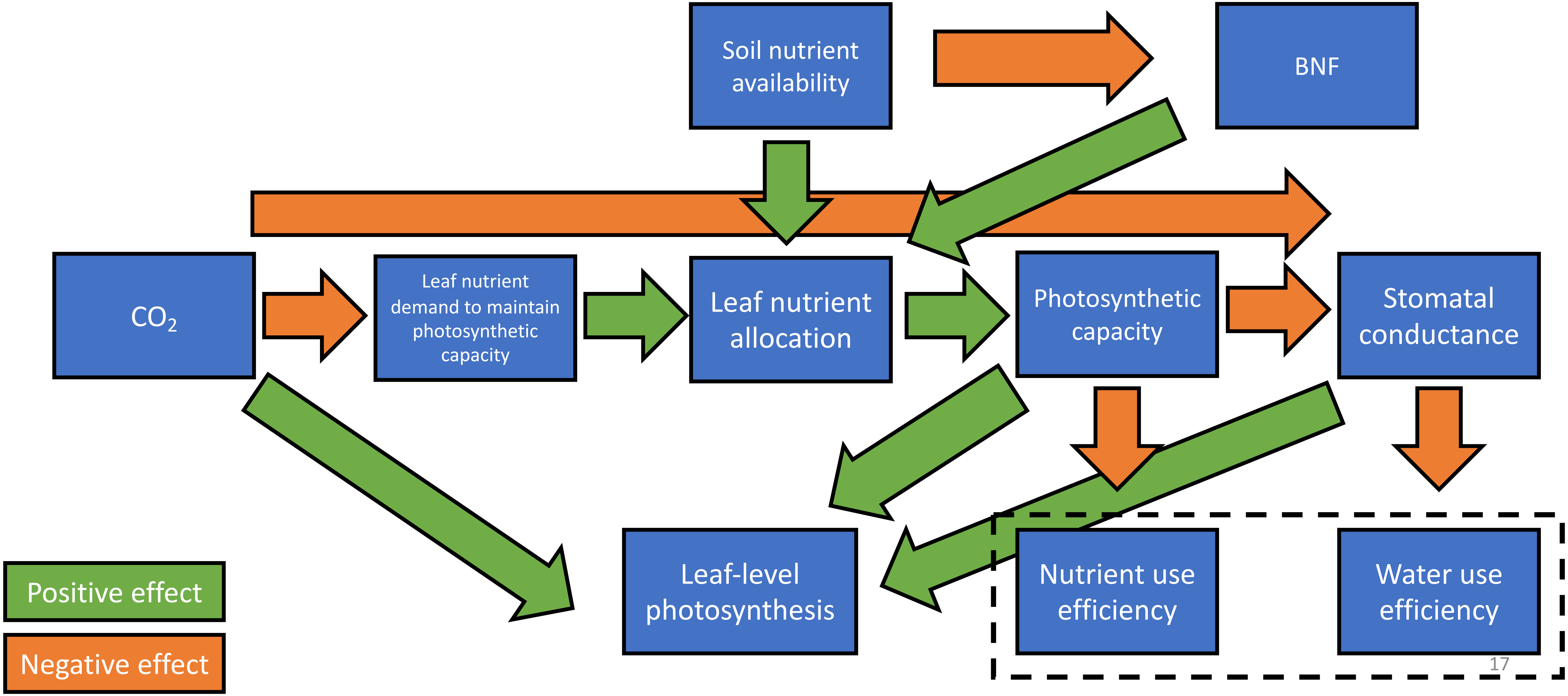
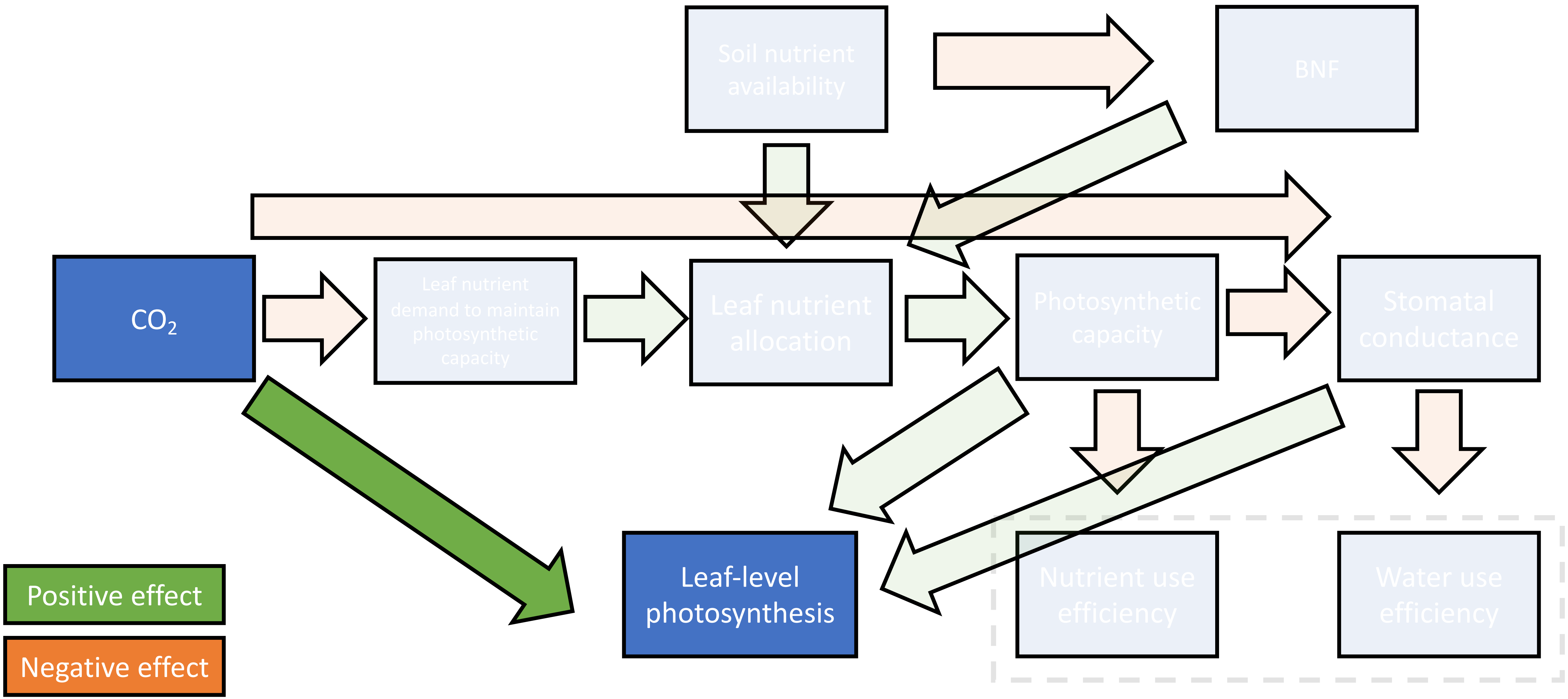


Inoculation with BNF should increase total leaf area and growth under low soil nutrients, but have similar total leaf area and growth under high soil nutrients

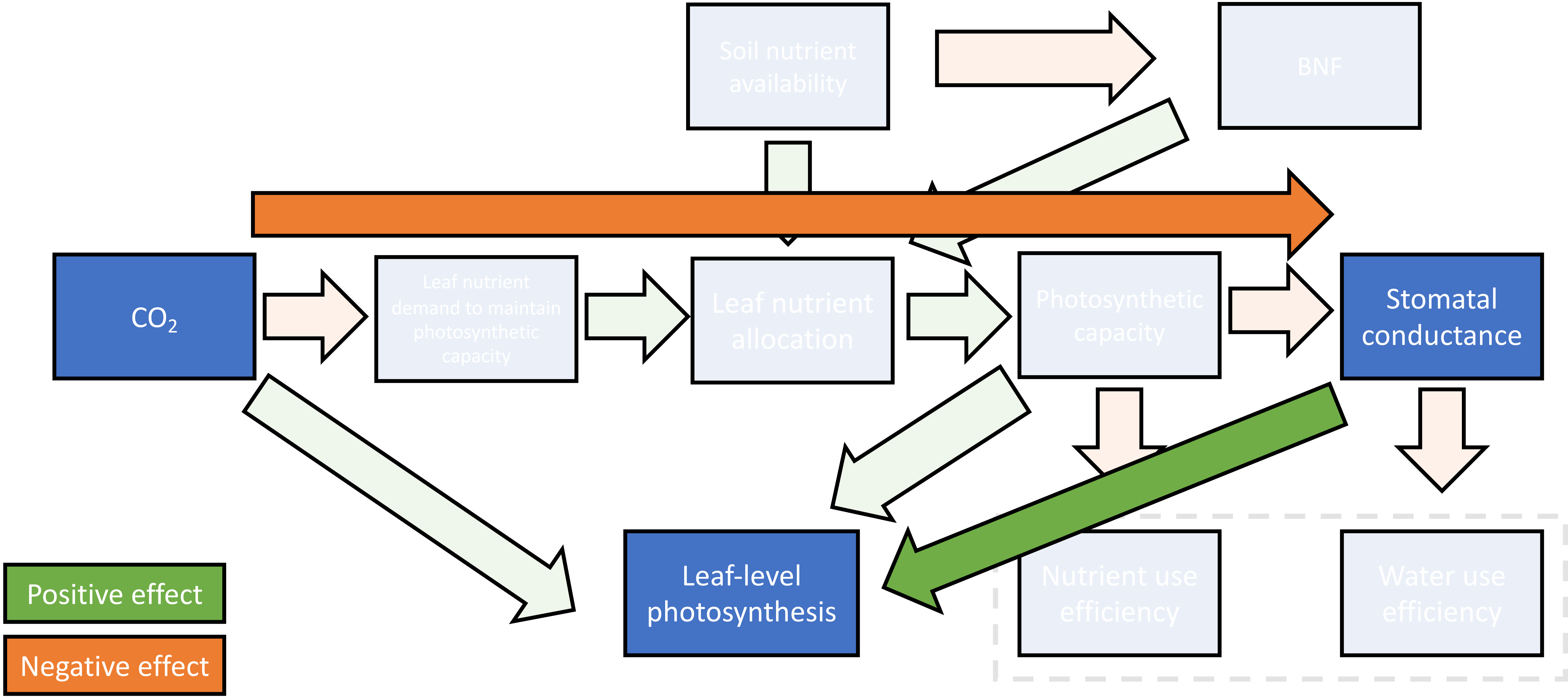
Hypothesis 2



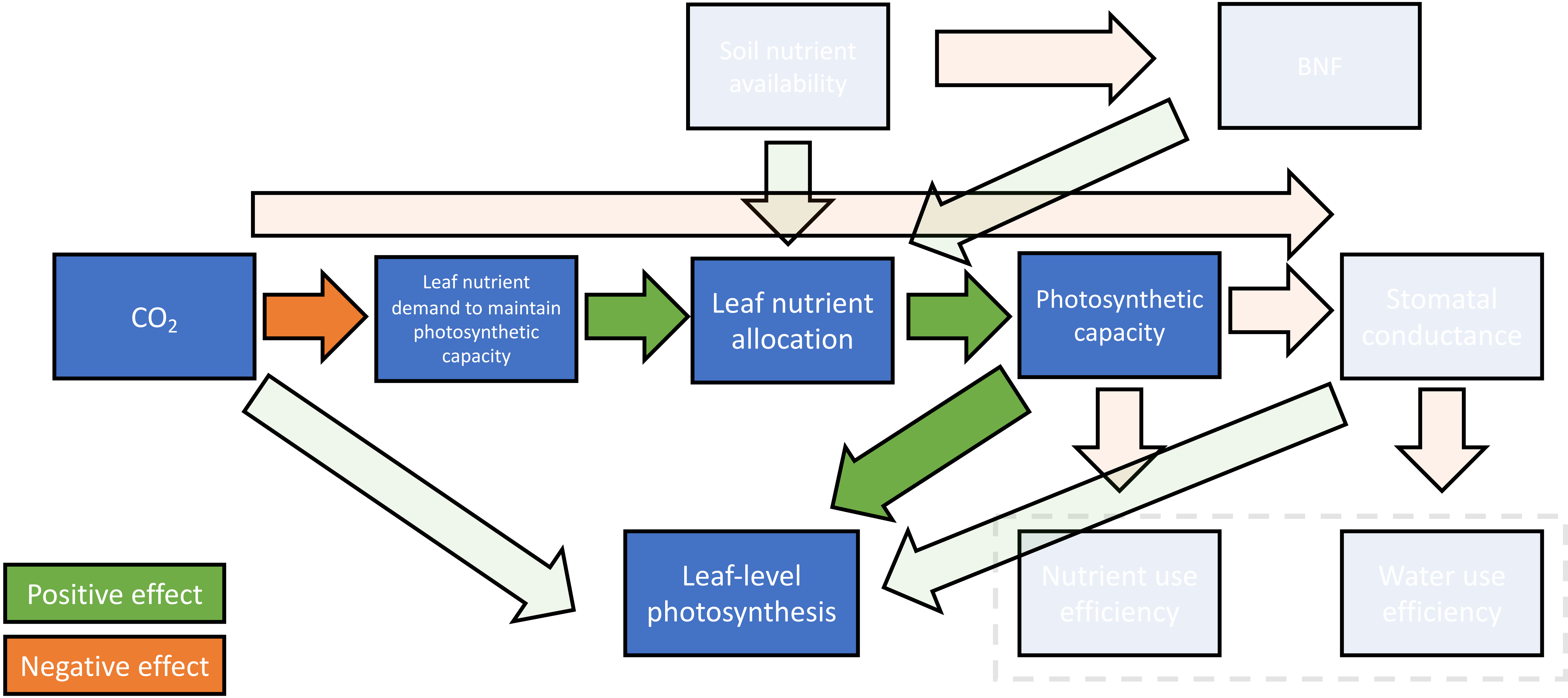
Hypothesis 2a: Increasing CO_2 will have a direct positive effect on leaf-level photosynthesis due to increased substrate needed to drive photosynthesis forward



Hypothesis 2b: Increasing CO_2 will decrease leaf-level photosynthesis as a function of reduced stomatal conductance



Hypothesis 2c: Increasing CO_2 will decrease leaf-level photosynthesis as a function of reduced maintenance costs for photosynthetic capacity



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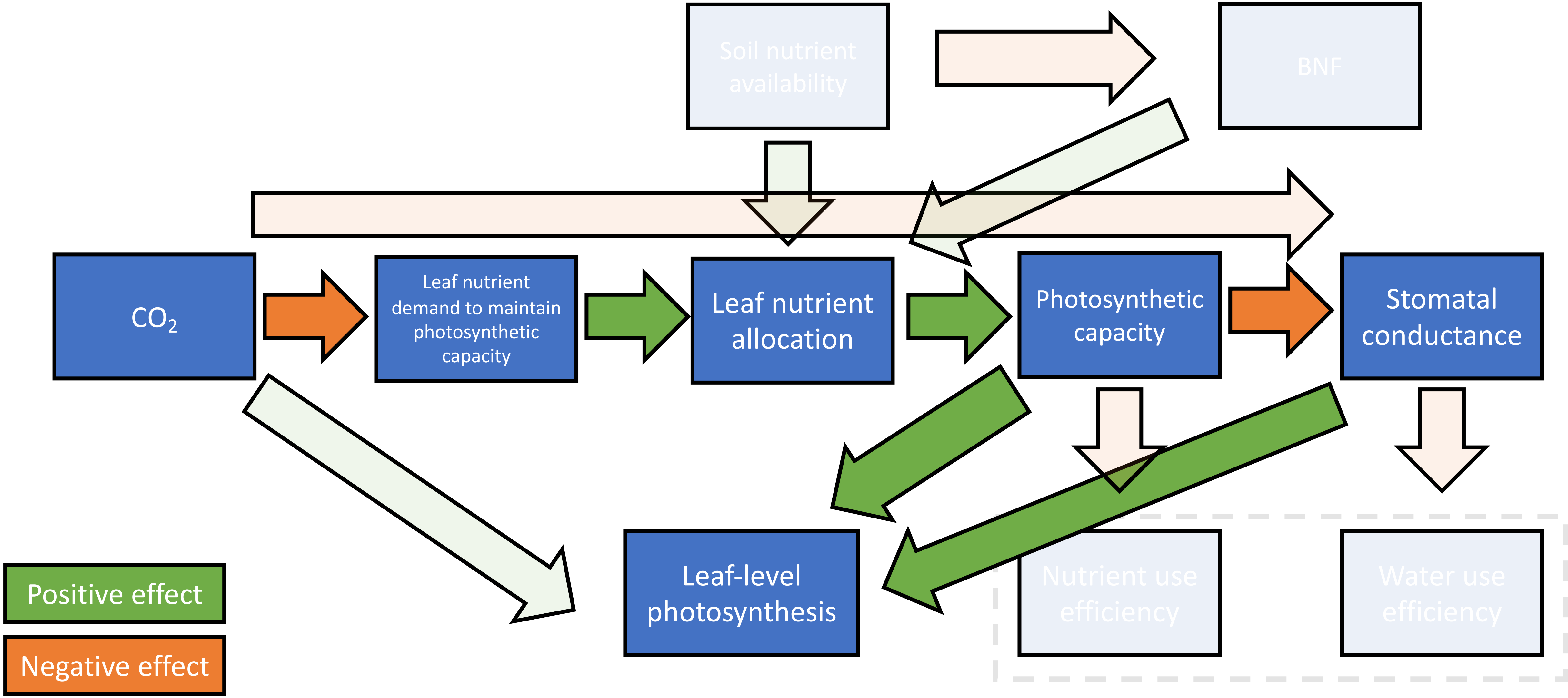

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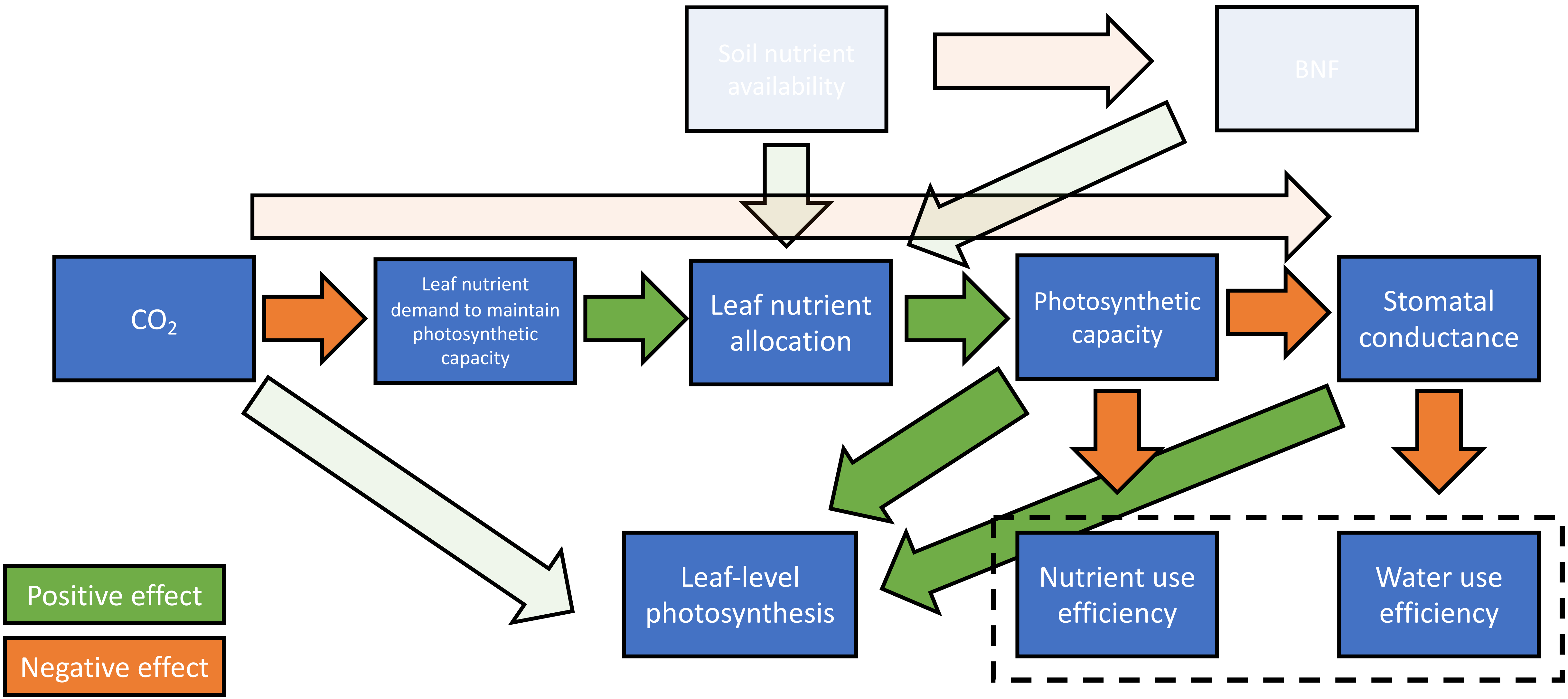

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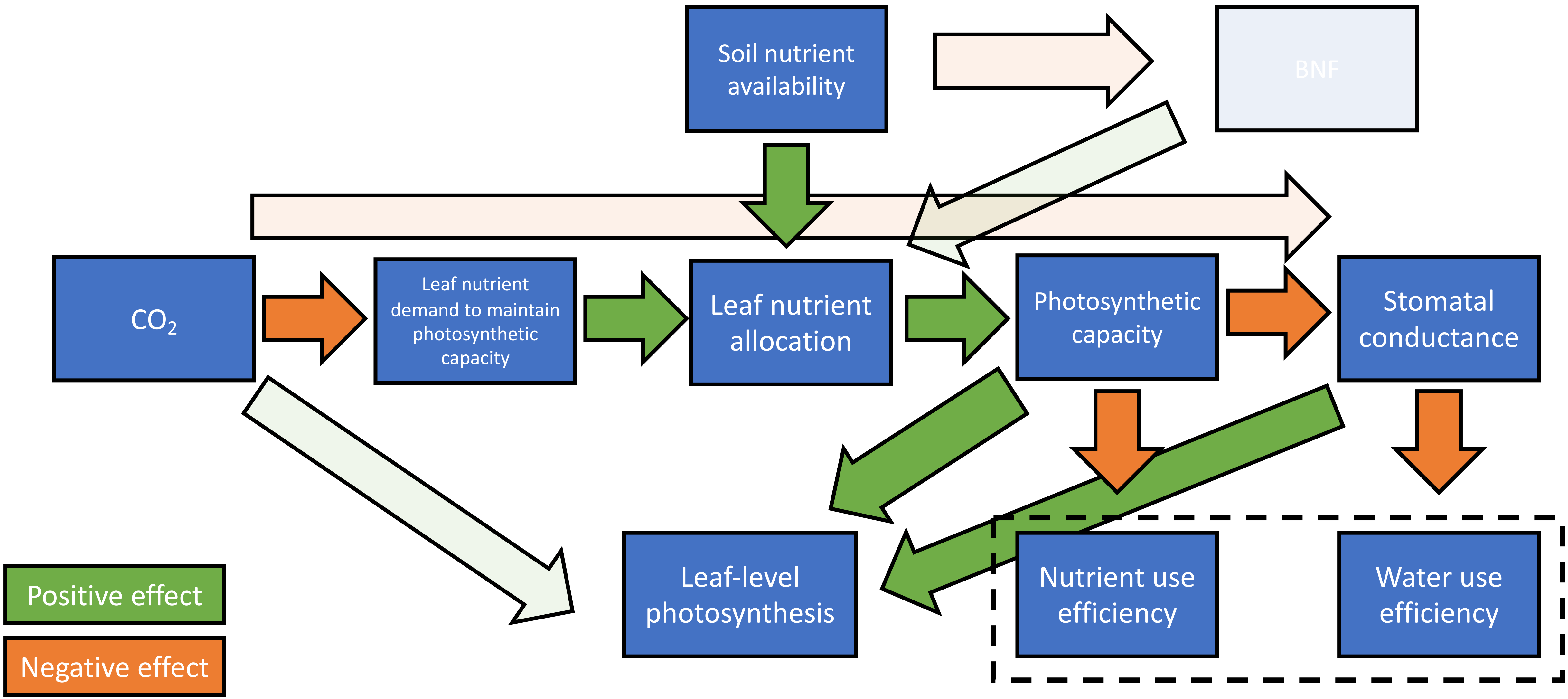

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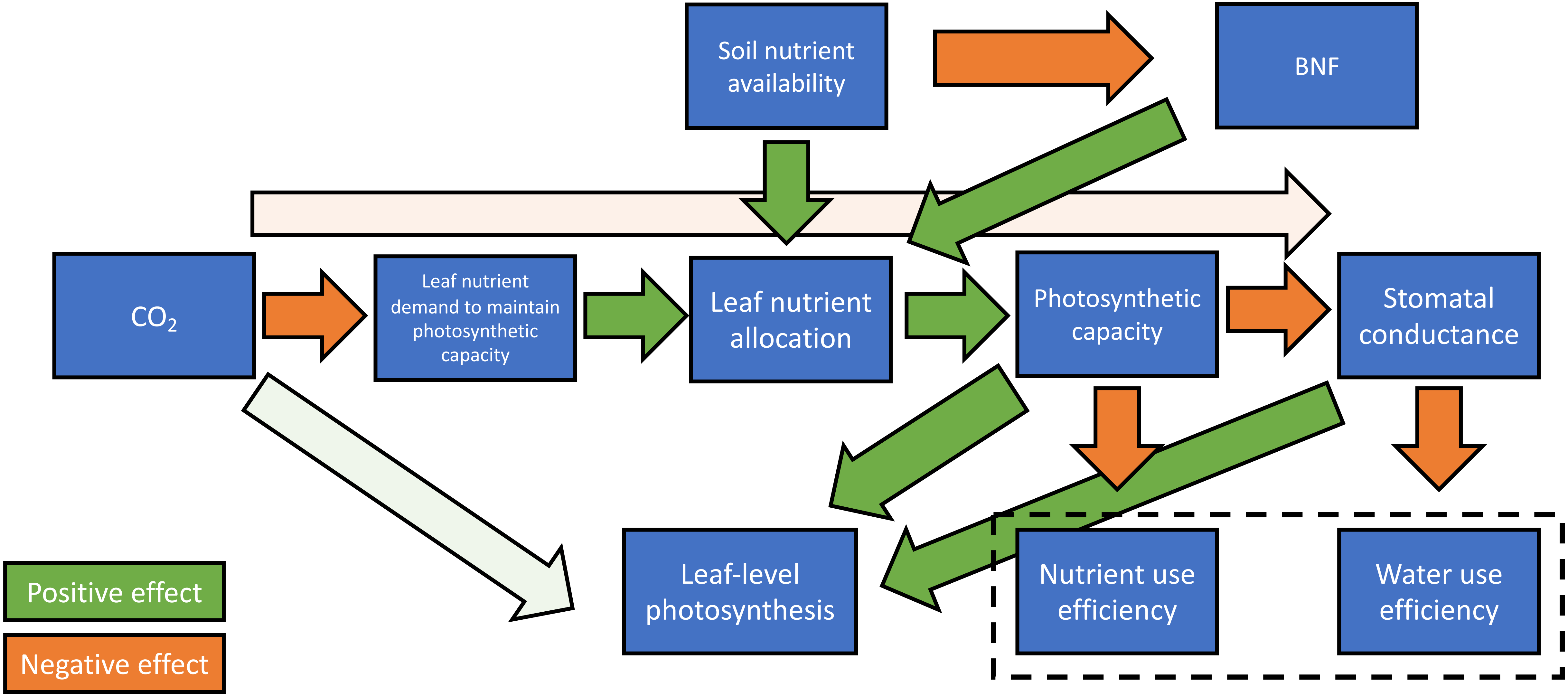
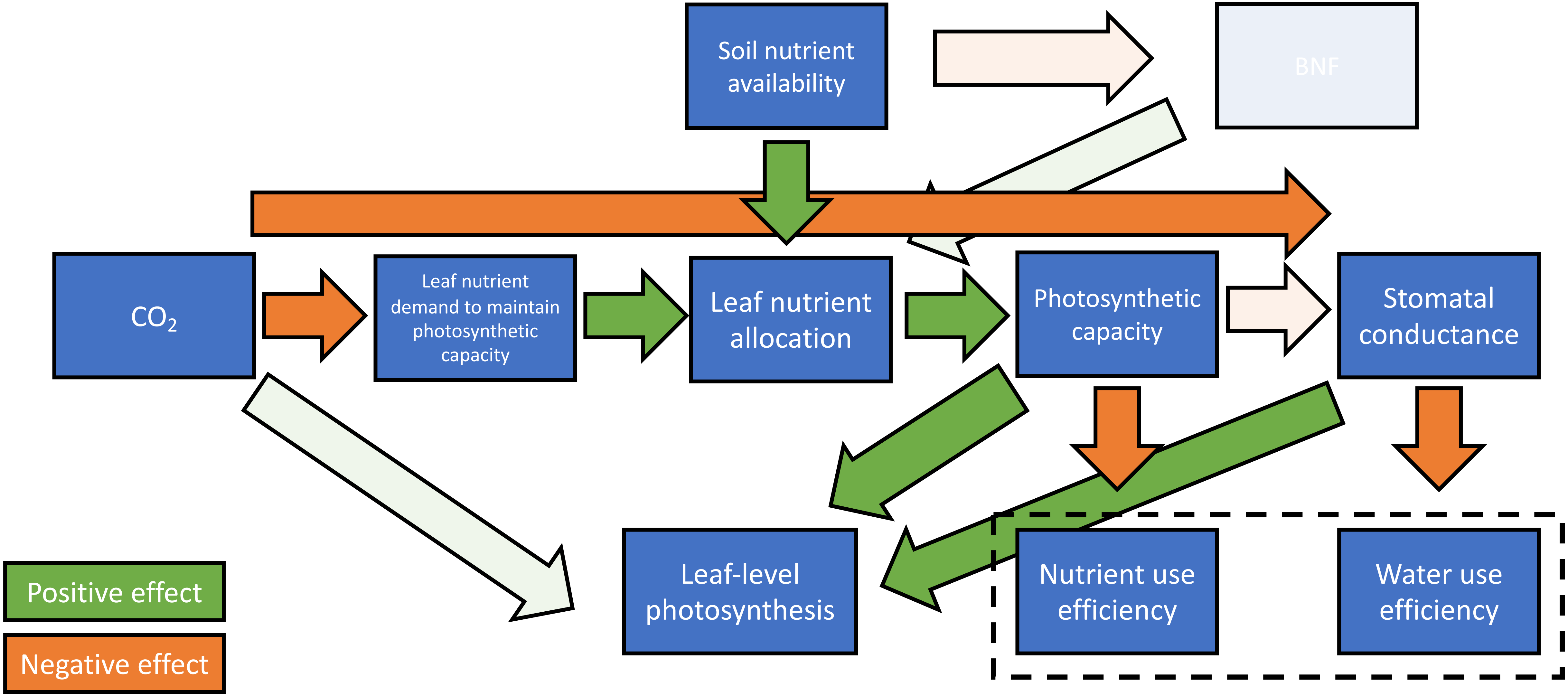


Hypothesis 2d: Increasing CO_2 will decrease leaf-level photosynthesis as a function of reduced maintenance costs for photosynthetic capacity <u>and</u> reduced stomatal conductance



Hypothesis 2