

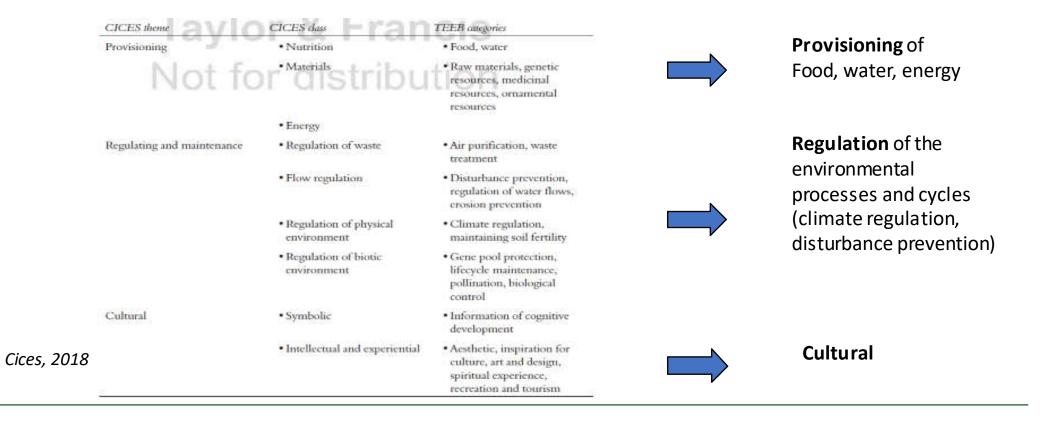
Improving transplant success: algae extracts and mycorrhiza

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Ecosystem services

Green areas provide ecosystem services = benefits arising from ecological processes which directly or indirectly increase human well-being



Tree planting programs



1000000

TREES PLANTED



Forestami

The benefits of one Million Trees^wLA

New EU Forest Strategy : planting three billion additional trees across Europe by 2030



As a flagship initiative of the **European Green Deal**, the European Commission has recently adopted the **New EU Forest Strategy**. **for 2030.**



Tree mortality after transplant can range between 7 and 34% within 5 years from planting (Koeser et al., 2014; Roman et al., 2014).

Was planning carefully done?

- Are there suitable locations to accommodate new plantings and resources for site amelioration?
- Are selected species and cv. available in nurseries in adequate quantity and quality?
- Are there resources available for postplanting care?



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Nursery pre-conditioning

Includes a series of cultural techniques applied during nursery production with the aim of producing sturdy plants, with superior tools to recover from transplant shock (Franco et al., 2006):

- Container size and typology
- Nursery substrates
- Deficit irrigation
- Fertilization management
- Root pruning
- Inoculation with mycorrhizal fungi
- Application of biostimulants and plant-growth regulators

HORTSCIENCE 45(12):1824-1829. 2010.

Effect of Container Design on Plant Growth and Root Deformation of Littleleaf Linden and Field Elm

Gabriele Amoroso¹, Piero Frangi, and Riccardo Piatti

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Effect of pot type and root structure on the establishment of *Tilia cordata* and *Ulmus minor* plants after transplanting

P. Frangi¹, G. Amoroso¹, R. Piatti¹, E. Robbiani¹, A. Fini² and F. Ferrini²

Nursery substrates

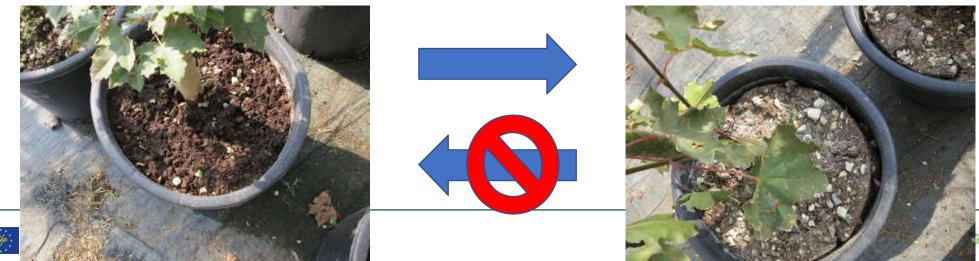


PNRR CN Biodiversity – National Biodiversity Future Centre (NBFC) – SPOKE 5: Urban biodiversity; Task 1.4: Plant production (2023–2025)

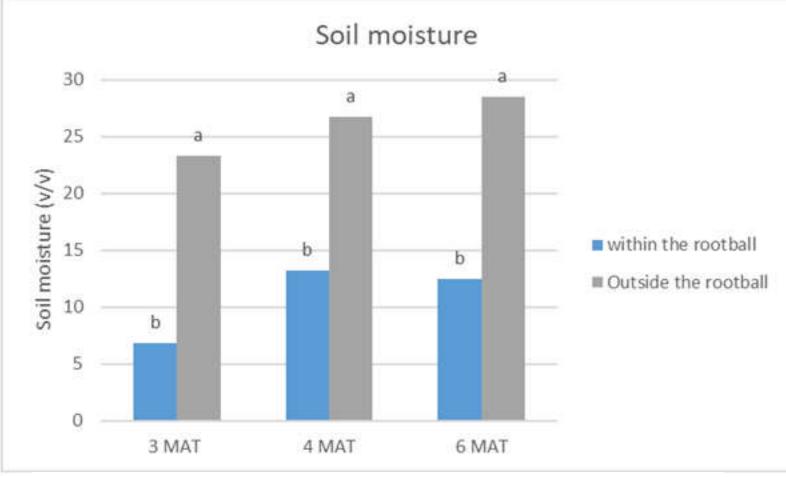
Aim: selection of environmentally friendly nursery substrate that:

- 1) Reduces the usage of peat
- 2) Shows similar performances as peat during nursery cultivation
- 3) Shows superior performances than peat after transplanting

Tested materials: wood fiber, zeolite, biochar, coconut



Rootball vs. soil moisture in newly transplanted trees from container





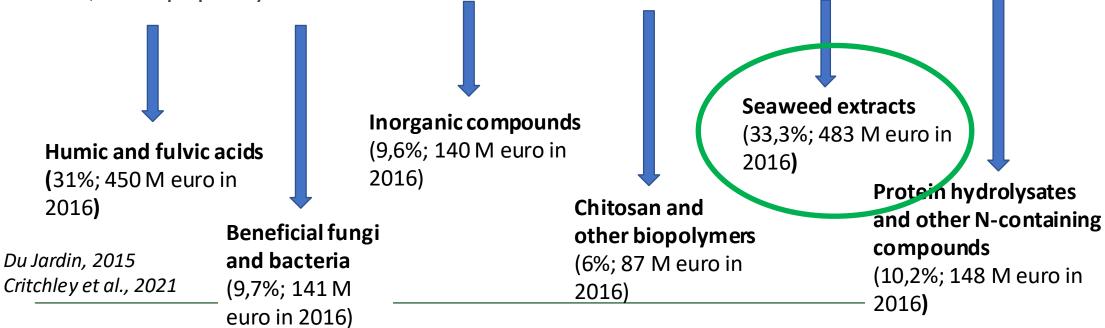
MAT = months after transplant. Transplant done at end February 2022

Biostimulants

A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content.

Diverse nature: single vs. mixed substances and microorganisms

Diverse physiological effects: improve nutrient efficiency and/or abiotic stress tolerance and/or crop quality traits



Seaweed extracts

Algae, especially brown-algae have been applied for agricultural purposes. *Ascophyllum nodosum* is most widely studied (Khan et al., 2009)

About 15 millions metric tons of algae-based products are manufactured annually (FAO, 2006)

Algae-extracts contain several bio-active ingredients:

Laminarin (glucan)			Fucoidal polysacc	n (sufurated haride)
Triggers plant defences				al activity in
Polyphenols	Plant	hormones	mamma	IS
Trigger plant defences	(cytok	inins, auxins)		
	Modul pathw	ate biosynthetic avs		Arginin
Mannitol (sugar alchool)	1			(N source)
Osmotic adjustment		Alginic acid		
Organic acids (D-mannuronic L-guluronic)	C;	Soil and rootin improvements, mycorrhiza	U	Inorganic ions (Na, Mg, K, Cl e SO4)
Water and nutrient uptake		ing corrinza		

Aims of experiment 1

1- Test the hypothesis that a seaweed extract can improve seedling growth, therefore fastening plant production process.

2- Test the hypothesis that a seaweed extract improves root morphological traits and root:shoot ratio, so that transplant success can be improved

3- Identify optimal dose of application



Experimental site

The experiment was carried out at ERSAF, in a regional forest nursery (Curno, BG, Italy), under a Cfb (Koppen-Geiger classification) climate.

Average temperature = 11.5 °C

Average rainfall = 1420 mm/year



The seaweed extract tested

Ascophyllum nodosum is a brown-algae which grows in coasts of Northern Europe, Iceland, Eastern Canada and Greenland with possible applications for horticulture

We used a pure extract (Agrofertil, France) made by grinding the algae at low temperatures. The extract was a powder

The extract was mixed with the nursery substrate at different rates

- D0: no extract (control)
- D1: 1 kg/m^3 (label dose)
- D2: 2 kg/m^3 (2x label dose)
- D3: 3 kg/m³ (3x label dose)



Umidità	12-15%	Carboidrati	45-60%
Proteine grezze	5-8%	Acido alginico	20-26%
Grassi grezzi	2-4%	Mannitolo	5-8%
Fibre grezze	>8%	Laminarina	2-6%
Materia minerale	40-55%	Fucoidano	8-10%
Sabbia	>0,5%	a conservation and	13 13
MACROELEMENTI		MICROELEMENT	<u> </u>
Azoto (N)	0,8-1,3%	lodio (I)	500-1200 ppm
Fosforo (P)	0,05-0,15%	Rame (Cu)	1-10 ppm
Potassio (K)	1-3%	Ferro (Fe)	150-1000 ppm
Calcio (Ca)	1-3%	Manganese (Mn)	0-50 ppm
Zolfo (S)	2-5%	Boro (B)	20-100 ppm
Magnesio (Mg)	0,5-0,9%	Molibdeno (Mo)	1-5 ppm
Cloro (Cl)	2-5%	Zinco (Zn)	40-200 ppm
Sodio (Na)	2-4%	Arsenico (As)	20-45 ppm
VITAMINE presenti	in quantità approssi	imative	
Carotene (A)	20-60 ppm	Tocoferolo (E)	50-200 ppm
Acido ascorbico (C)	2000-1000 ppm	Niacina (B3)	10-30 ppm
Riboflavina (B2)	5-10 ppm	B12 ca. (M)	0,004 ppm
Tiamina (B1)	1-5 ppm	Acido folico (B9)	0,2 ppm
Biotina (B)	0,1-0,4 ppm	Acido folinico	0,2 ppm

sperimentazione (agrofertil.fr).

Plant material and growing conditions

- Seeds of *Carpinus betulus, Crataegus monogyna, Fagus sylvatica* were obtain from local certified seed forests.
- Seeds were cleaned using sodium hypochlorite and stratified in moist sand at 4 °C to overcome dormancy
- In Feb. 2021, seeds were seeded in 0.4 L plastic trays, according to a randomized block design with 10 blocks (32 plants per species and seaweed treatment per block, 1280 plants per species; 3840 plants in total)
- A commercial substrate specifically developed for seedlings (Hochmoor, Teflor, Italy). The substrate contains with 1 kg/m3 of soluble fertilizer and 1 kg/m3 a controlled release fertilizer, was used.



Substrate component or trait	Amount (v/v) or value
Brown peat + montmorillonite	30%
Blond peat (10-20 mm)	50%
Coconut fiber	20%
рН	6.0
EC	0.3 dS/m
Density	100 kg/m ³
Porosity	95%

Plant material and growing conditions

In Jan. 2022, plants were re-potted into 1.7 L containers and grown under a tunnel under full sunlight for one additional growing season.

Algae extracts were not applied at repotting

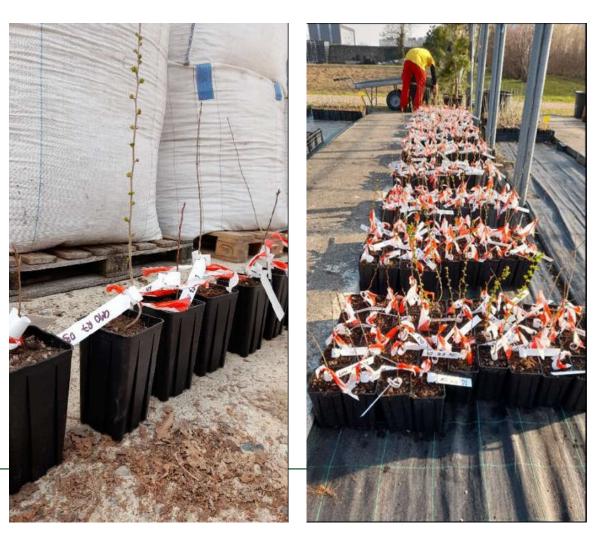
After repotting, plants were arranged according to a randomized block design with 10 blocks (2 plants per treatment and species in each block; 240 plants in total)

Irrigation was carried out daily (10-15 minutes during nighttime)

No additional fertilization other than that included in the substrate was done

Weeding was conducted manually, twice per month

No pesticide was applied



Measurements

Growth traits (measured on 2, 6, 12, 24, 48 and 72 weeks after emergence):

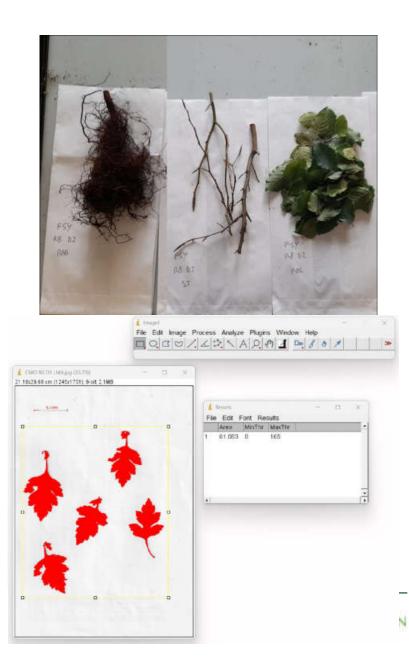
1- plant height (cm): measured from the flare to the base of the apical bud

2- leaf, stem, and root dry weights (g): measured after cutting the stem at the flare, separating the leaves, cleaning the roots with a flush of air, and oven-drying leaves, stems, and roots separately at 70°C until constant weight

3- Average and Total leaf area (cm²): measured scanning all plant leaves with an A3-scanner

4- Root:shoot: calculated as DWroots/(DWstem + DWleaves)

5- Relative Growth Rate of the plant and of roots: calculated as (InDWt1 - InDWt0)/(t1-t0)



Measurements

Root traits (measured on 8, 13, 23, 58, and 70 weeks after emergence):

1- Total root length (cm): measured using the root-line intersect method (Tennant, 1975)

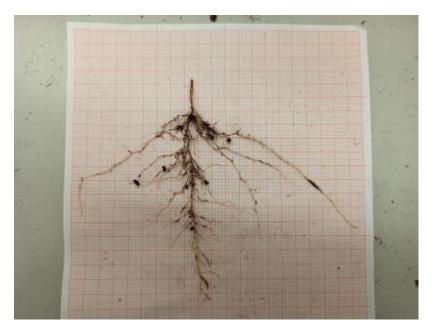
2-Rootdensity(cm/dm3): calculated as total root length/effective container volume

3- Specific root length (cm/g): root length per unit weight.

Physiology (measured on 2, 6, 12, 24, and 72 weeks after emergence):

- 1 Net CO₂ assimilation (μ mol m⁻² s⁻¹)
- 2- Stomatal conductance (mmol m⁻² s⁻¹)
- 3- Sub-stomatal and chloroplastic CO2 concentration (ppm)
- 4- Water use efficiency: calculated as A/E

5- Leafgreenness index: measured using a SPADmeter

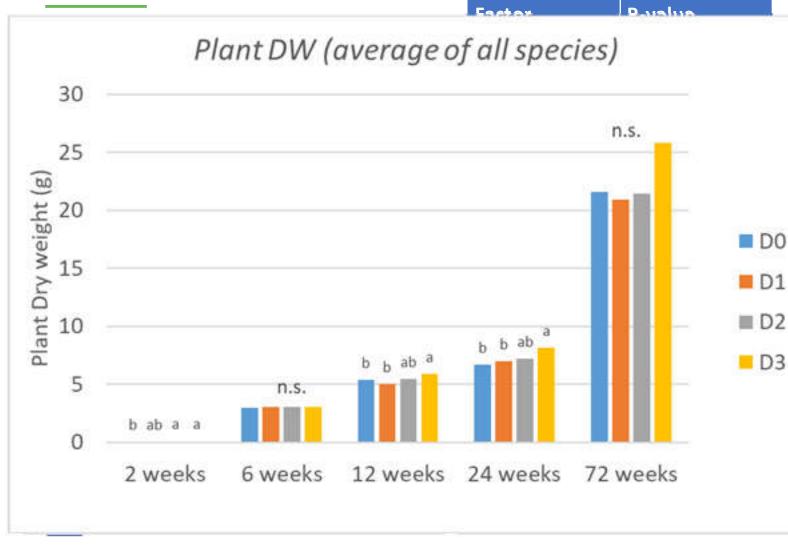






All data were analysed using mixed models tool in SPSS. Homogene ous subsets were separated using Sidak test

Relative growth rate: whole plant

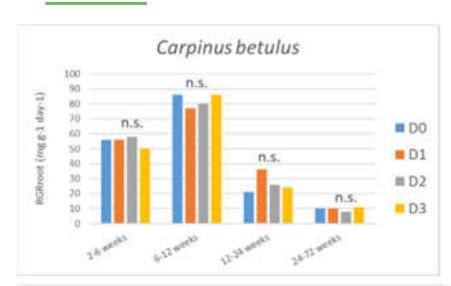


 It indicates the amount of dry biomass produced per unit existing g DW per day

Higher RGRplant was observed in the 2 weeks after emergence in all species RGRplant <u>transiently</u> increased when biostimulants werea applied at 2x or 3x label doses, compared to control Larger effect in *F. sylvatica*



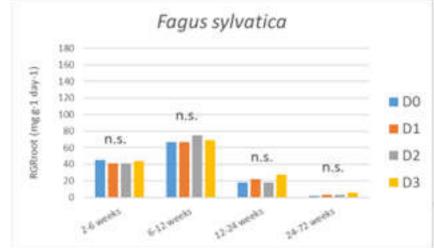
Relative growth rate: roots

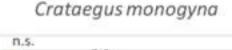


P-value
**
n.s.
**
n.s.
**
n.s.
n.s.

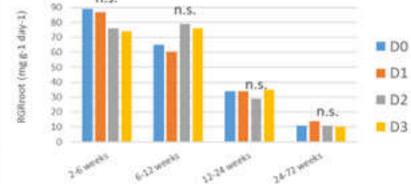
- It indicates the amount of root dry biomass produced per unit existing g DW per day
- Root RGR could not be calculated in between germination and 2 weeks
- Biostimulant application did not promote root RGR





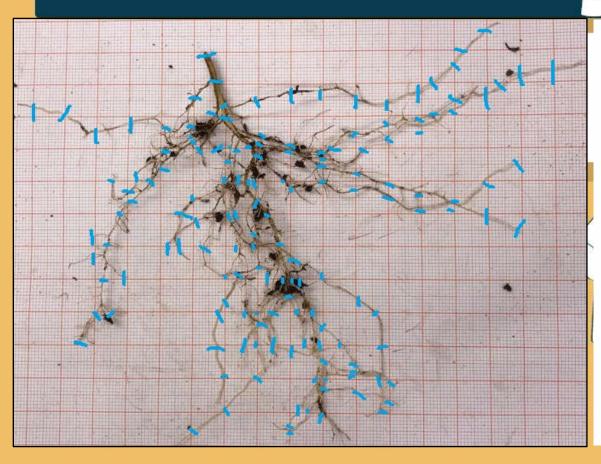


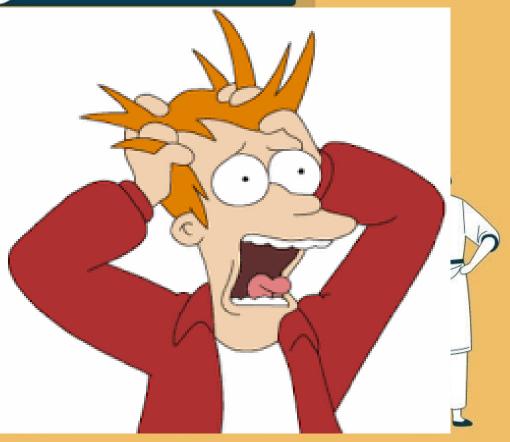
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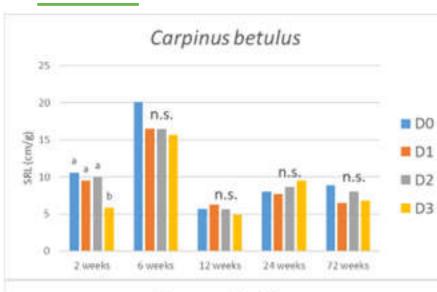


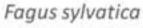
Going beyond root weight

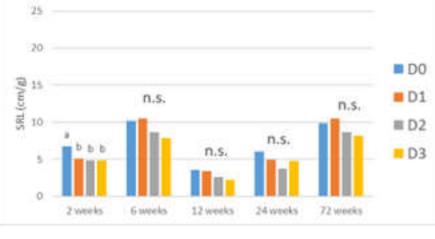




Specific root length

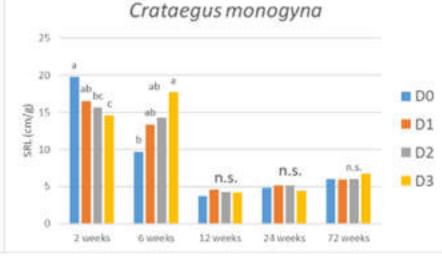


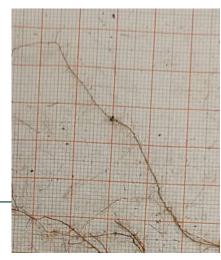




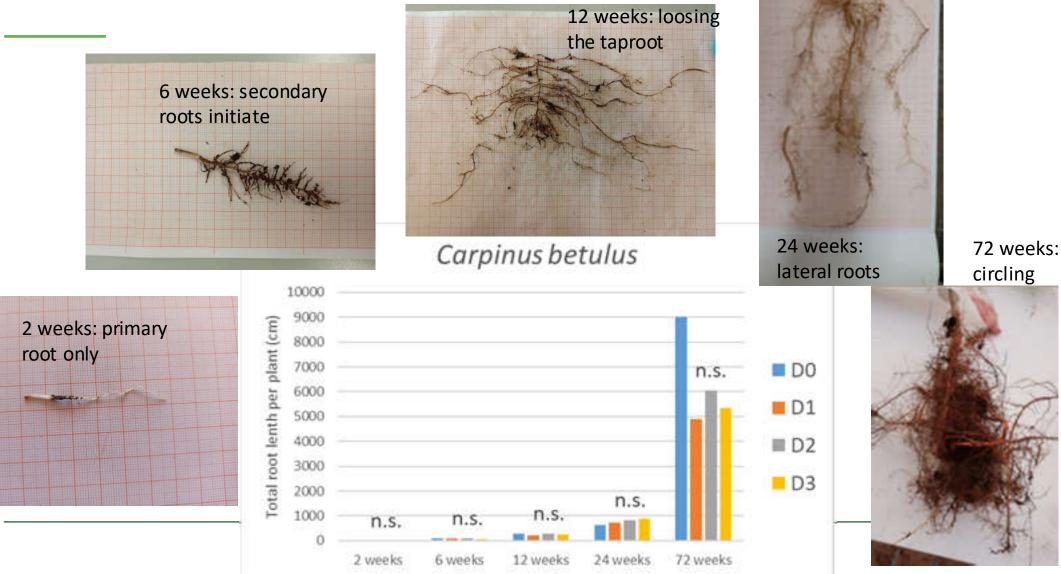
Factor	P-value
Pspecies	**
Pdose	n.s.
Ptime	**
PspeciesXdose	n.s.
PspeciesXtime	**
PdoseXtime	*
PspXdoseXtime	n.s.

- It indicates the total length of 1 g of root dry biomass
- SRL decreased in all species from 6 to 12 weeks (root secondary growth)
- *Fagus* had shorter roots per unit root diameter than other species
- Algae reduced SRL 2 weeks after emergence, then differences disappeared



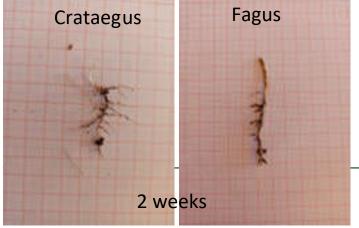


Total root length - Carpinus



Total root length



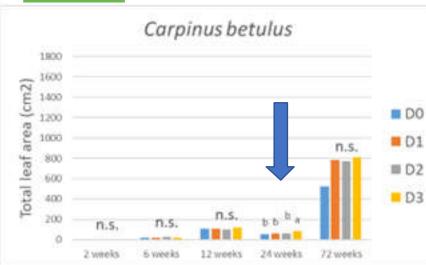


- Total root length increased with species' sunlight requirements (*Crataegus* > *Carpinus* ≥ *Faqus*)
- Biostimulants did not consistently affect total root length per plant



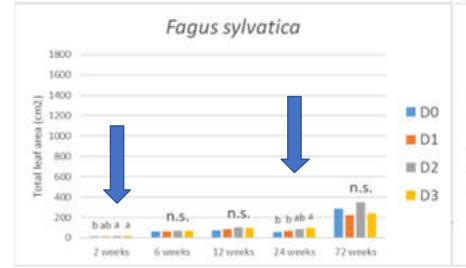


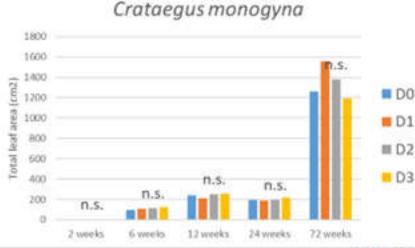
A look above-ground: Leaf Area



Factor	P-value
Pspecies	**
Pdose	n.s.
Ptime	**
PspeciesXdose	n.s.
PspeciesXtime	**
PdoseXtime	n.s.
PspXdoseXtime	n.s.

- F. Sylvatica had higher TLA than other species 2 weeks after emergence. Thereafter, C. monogyna had higher leaf area than other species
- Biostimulants transiently increased TLA when applied at 3x label dose
- Effects were mostly apparent in fall and may be due to enhanced tolerance to summer heat or delayed stress-induced leaf senescence (polyphenols?)







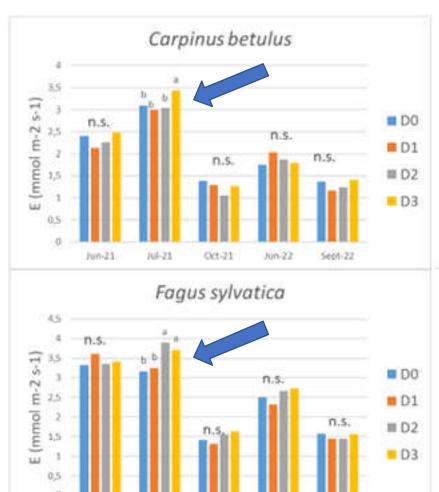
Leaf gas exchange

Sometimes measuring leaf gas exchange is pleasant...

...not this time!



Leaf gas exchange: transpiration



0:1-21

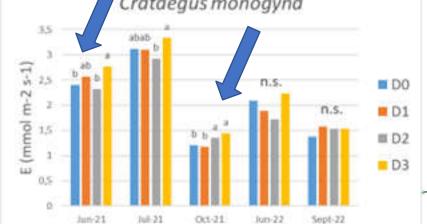
Jun-22

5401-22

Jun-21

344-21

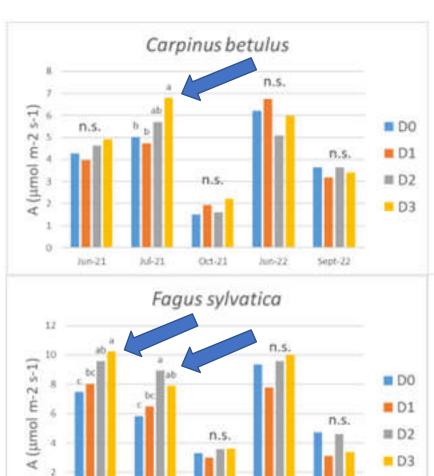
Factor	P-value
Pspecies	**
Pdose	**
Ptime	**
PspeciesXdose	n.s.
PspeciesXtime	**
PdoseXtime	n.s.
PspXdoseXtime	*



- Mannitol in algae-extracts may have improved osmotic adjustment and water uptake.
- Seaweed extracts transiently increased the amount of water transpired per unit leaf area compared to control.
- The effect was significant only in the 1st year and only when algae were applied a 3x label dose



Leaf gas exchange: net CO2 assimilation



Oct-21

Jun-22

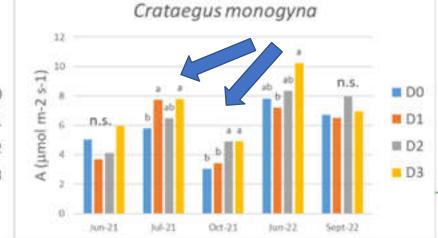
Sept-22

0

Jun-21

345-21

Factor	P-value
Pspecies	**
Pdose	**
Ptime	**
PspeciesXdose	*
PspeciesXtime	**
PdoseXtime	*
PspXdoseXtime	n.s.



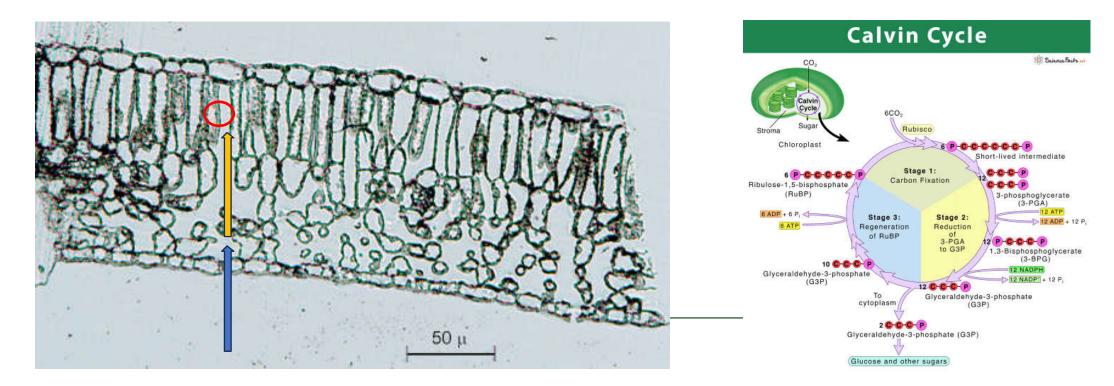
- The amount of CO2 assimilated by 1 m2 leaf area was increased when seaweed extracts were applied at 3x label dose, compared to control.
- Such effect was observed within 1 year since biostimulant application



Leaf gas exchange: photosynthetic limitations

To be converted into carbohydrates, CO₂ must:

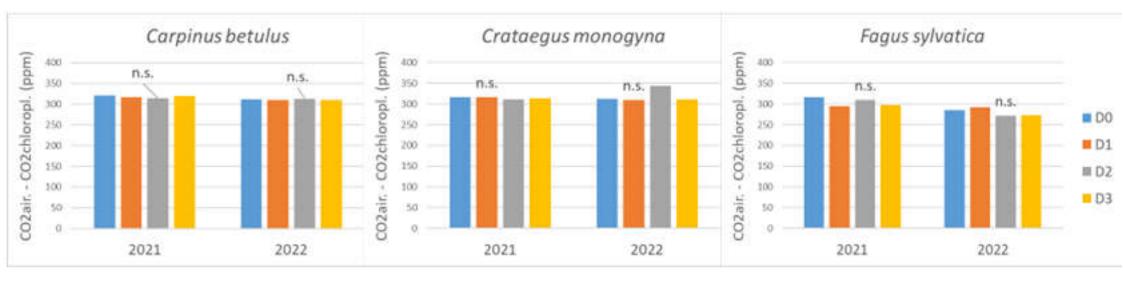
- 1- diffuse from the outer air to the leaf (stomata, blue arrow)
- 2- diffuse from the substomatal chamber to the chloroplast (mesophyll, orange arrow)
- 3- be carboxylated (Rubisco and Ribulose, red dot)



Leaf gas exchange: diffusive limitations (stomata + mesophyll)

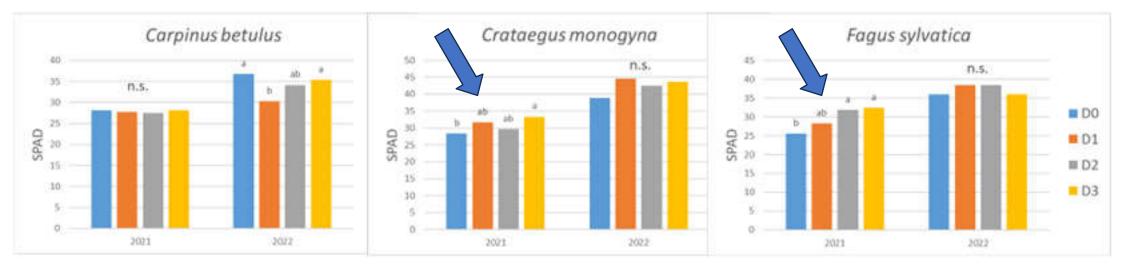
Stomatal and mesophyll factors determine diffusive limitations to photosynthesis, which can be estimated as (Loreto et al., 1994):

• Diffusive limitations = CO2 air - CO2 chloroplasts (the lower it is, the easier the diffusion)



• Diffusive limitation were not affected by seaweed extracts

Leaf gas exchange: leaf greenness index



- Biostimulants at 3x label dose increased leaf greenness index in 2 of the 3 species, during the 1st year
- Short-term effect.
- Nitrogen involved? (A. nodosum has up to 1.3% N which can yield about 40 g N/m3 substrate when it is applied at 3x label dose; comparable to several organic fertilizers, e.g. compost, horse and bone meal manure)

Experiment 1: take home message

- *A. nodosum* improved growth in the short-run during nursery cultivation. This was partly due to higher chlorophyll content in plants treated with algae at high doses which determined a short-term increase in photosynthetic rate
- Annual applications of *A. nodosum* may fasten plant production process
- The optimal dose of application was 3x label dose = 3 kg/m3
- Little evidences were found that its application may have long-term effects that promote tolerance to transplanting shock.





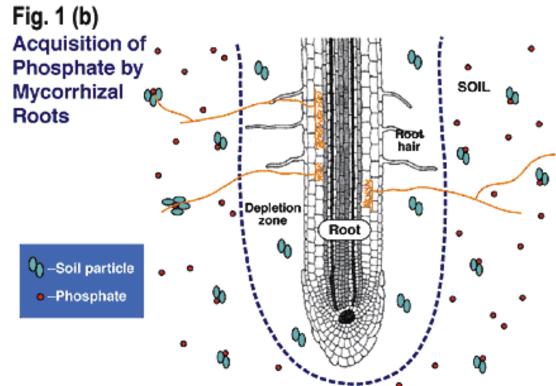


Mycorrhizae-forming fungi are ecologically significant because they form relationships in and on the roots of a host plant in a mutualistic association

• Improved P-uptake (with GvPT and GiPT, specific AMF trasporters and down-regulation of direct uptake by plant) and ability to absorb P outside of the depletion zone (*Karandashov and Bucher, 2005, Trends in plant Science*)

• Protection against drought and salinity (*Augè, 2000, Mycorrhiza*)

• Protection against pathogens (Tygensen et al., 2004, Eur. J. Plant Pathol.)



Mycorrhizal inoculation of young plants in the nursery requires little inoculum (25-50 ml per tree; up to 20 plants per L inoculum) but:

1- Inocula must survive standard nusery cultivation practices and substrates

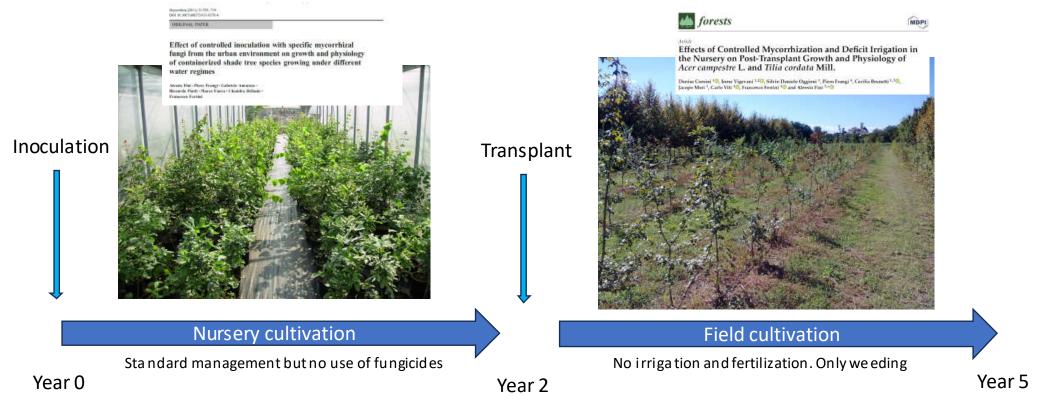
2- Inocula must keep functionality after transplanting (competition with native soil microbiota)

The aim of experiment 2 was:

• To evaluate if inoculation with specific mycorrhizal strains in the nursery can build a long-term mutualism which enhances tolerance to transplanting stress.

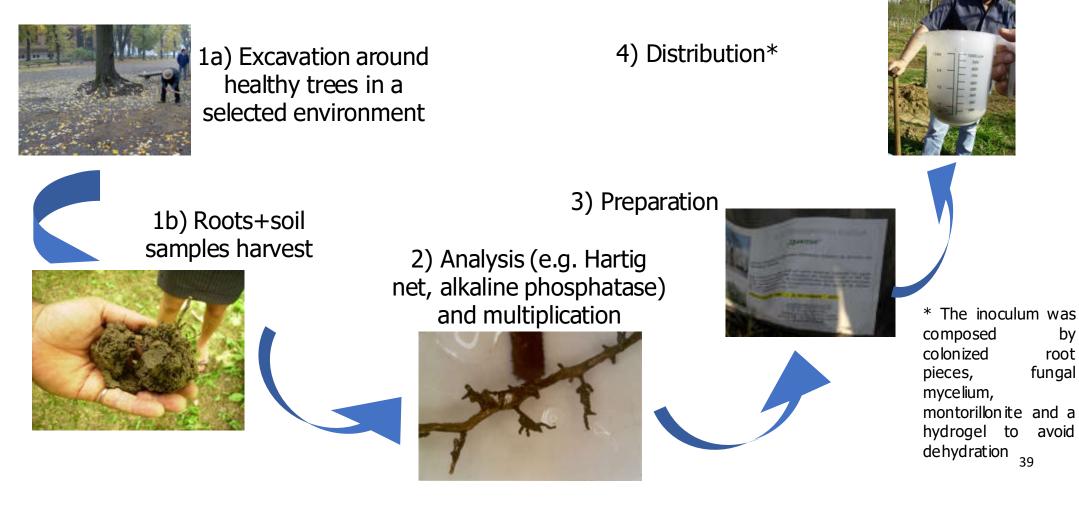
Fondazione Minoprio

• 160 2-year-old Acer campestre and Tilia cordata plants were used.



Selection of the inoculum

Instead of using commercial products, a controlled mycorrhization approach was used (overall, it took about 8 months to get the inoculum)



Selection and distribution of the inoculum

STEP 2: Selection

Based on laboratory analyses, strains of the following species were selected:

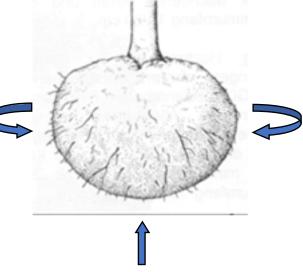
A. campestre: native strains of Glomus geosporum, G. mossae, and G. clarum (all VAM)

T. cordata: native strains of Boletus edulis (ECM) and Glomus mossae (VAM).

STEP 4: Distribution

It was done at potting, in March 2008 on half of the plants (+I), while the other half was not inoculated (-I).

Every 10 cm of stem diameter correspond to 450-500 ml of inoculum.



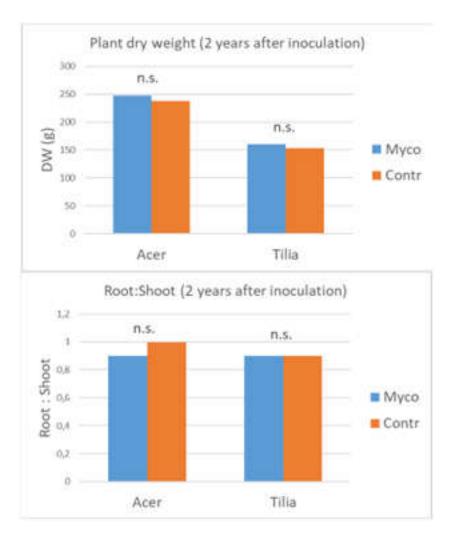
- 2/3 spreading on the sides
- 1/3 putting into bottom

Phase 1: nursery cultivation

Percentage of mycorrhizal root tips (ECM) and root colonization after staining (AMF) measured using a stereomicroscope after 7 months of cultivation in inoculated (+I) and non-inoculated plants. Different letters denote significant differences between +I and –I plants at p<0,05

Species	Inoculation (I)
	+I (%)	-I (%)
Maple	53 a	24 b
Linden (ECM)	81 a	59 b
Linden (AMF)	17 a	10 b

- -I plants were also mycorrhizal
- Inoculation increased mycorrhizal root colonization



Growth of woody organs and root:shoot were not affected by inoculation

Phase 1: nursery cultivation

Vcmax and Jmax are photosynthetic parameters obtained from A/Ci curves which represent Rubisco and electron transport activity in the photosystems.

Different letters denote significant differences between +I and –I plants at p<0,05

Ksp, Ksx, and KI denote whole plant, soil to xylem, and leaf conductivity to water. They are crucial parameters to understand the hydraulic functionality of the plant. Different letters denote significant differences between +I and –I plants at p<0,05

	Inoculation (I)		Species	Parameter	Inoculation (I)	
	+I	-1	<u> 22</u>		+I	-I
			Maple	$K_{\rm sp} \times 10^{-7} ({\rm m \ s^{-1} \ MPa^{-1}})$	78.9 a	84.1 a
V _{cmax}	29.1 a	16.0 b		$K_{\rm sx} \times 10^{-7} ({\rm m \ s^{-1} \ MPa^{-1}})$	169.0 a	164.1 a
J _{max}	72.5 a	36.5 b		$K_1 \times 10^{-7} (\text{m s}^{-1} \text{ MPa}^{-1})$	178.2 a	140.5 a
	43.0 a	24.4 b	Linden	$K_{\rm sp} \times 10^{-7} ({\rm m \ s^{-1} \ MPa^{-1}})$	155.0 a	115.6 b
				$K_{\rm sx} \times 10^{-7} ({\rm m \ s^{-1} \ MPa^{-1}})$	402.9 a	351.0 a
Jmax	00.0 a	40.7 0		$K_1 \times 10^{-7} \text{ (m s}^{-1} \text{ MPa}^{-1}\text{)}$	327.1 a	195.9 b
					Fini et	t al., 2011
	V _{cmax} J _{max} V _{cmax} J _{max}	$J_{\max} = 72.5 a$ $V_{\max} = 43.0 a$	V_{cmax} 29.1 a16.0 b J_{max} 72.5 a36.5 b V_{cmax} 43.0 a24.4 b	V_{cmax} 29.1 a 16.0 b Maple J_{max} 72.5 a 36.5 b Linden V_{cmax} 43.0 a 24.4 b Linden	V_{cmax} 29.1 a 16.0 b Maple $K_{sp} \times 10^{-7} \text{ (m s^{-1} MPa^{-1})}$ J_{max} 72.5 a 36.5 b $K_1 \times 10^{-7} \text{ (m s^{-1} MPa^{-1})}$ V_{cmax} 43.0 a 24.4 b Linden $K_{sp} \times 10^{-7} \text{ (m s^{-1} MPa^{-1})}$ V_{cmax} 68.8 a 40.7 b $K_{sx} \times 10^{-7} \text{ (m s^{-1} MPa^{-1})}$	+I-I V_{cmax} 29.1 a16.0 b J_{max} 72.5 a36.5 b V_{cmax} 43.0 a24.4 b J_{max} 68.8 a40.7 b

- Activity of photosynthetic enzymes was higher in +I than in -I plants of both species
- Inoculation increased plant conductivity in linden



Then, transplant!

- Plants were arranged according to a randomized block design with 6 blocks and 6 plants per species and mycorrhizal treatment in each block (144 plants in total)
- Plants were planted into hand-dug planting holes (as deep as the rootball and 1.5 times the width of the rootball) following a 3 x 3 m planting design
- Soil of the experimental site is a loam (51% sand, 40% silt and 9% clay) and sub-acid (pH 6.41), with high levels of gravel (16%) and available phosphorus (317 mg kg⁻¹), a normal content of organic matter (18 g kg⁻¹) and total N (1.30 g kg⁻¹) and a low CEC (5 meq 100 g⁻¹)



Measurements after transplant

- **Root colonization**: assessed 34 months after transplanting (MAT).
- **Growth and mortality**: both plant dry weight (measured 34 MAT) and shoot extension (measured 11, 23, and 34 MAT) were used to quantify the effects on inoculation on growth. Mortality was assessed as the frequency of dead plants
- Leaf gas exchange: measured 4, 5, 14, 16, 18, 27, 28 and 30 MAT using an infrared gas analyzer (Ciras-2, PP-System)
- Water relations and leaf soluble carbohydrates: measured 5, 14, and 28 MAT. A pressure bomb was used to measure pre-dawn, xylem, and midday water potentials. HPLC-RI was used to quantify individual carbohydrates



Detailed methods are reported in ^{Article} Effects of Controlled Mycorrhization and Deficit Irrigation in

Effects of Controlled Mycorrhization and Deficit Irrigation in the Nursery on Post-Transplant Growth and Physiology of *Acer campestre* L. and *Tilia cordata* Mill.

MDPI

Denise Corsini ¹⁽⁰⁾, Irene Vigevani ^{1,2}⁽⁰⁾, Silvio Daniele Oggioni ³, Piero Frangi ⁴, Cecilia Brunetti ^{1,5}⁽⁰⁾, Jacopo Mori ¹, Carlo Viti ¹⁽⁰⁾, Francesco Ferrini ¹⁽⁰⁾ and Alessio Fini ^{3,*}⁽⁰⁾

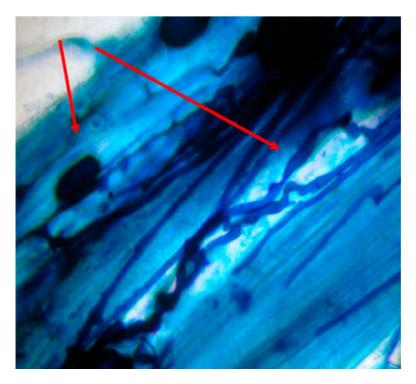


- Species were independently analyzed for all the measured parameters because of the different tested inoculants applied to species
- Dendrometric and physiological parameters were analyzed on each individual sampling date using General Linear Model (GLM; SPSS 16.0, SPSS Inc.)

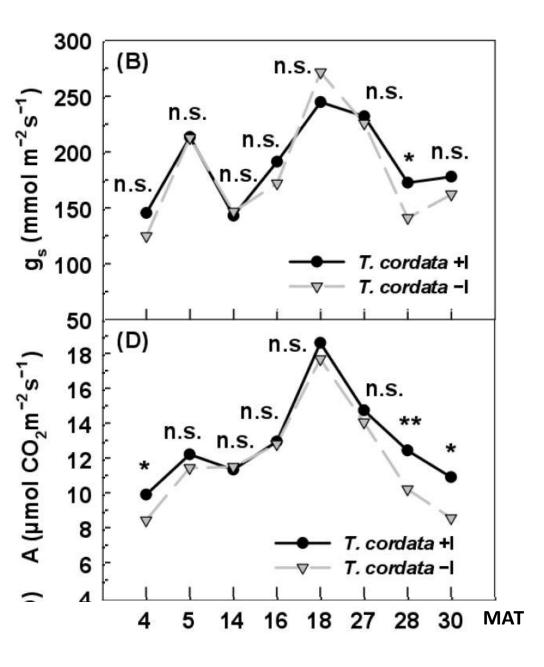
Root colonization (34 months after transplanting)

Percentage of mycorrhizal root tips (ECM) and root colonization after staining (AMF) measured using the magnified intersection method in inoculated (+I) and non-inoculated plants. Different letters denote significant differences between +I and –I plants at p<0,05

	Inoculation (I)		
Species	+I (%)	−I (%)	
Acer campestre (AMF)	70.6 a	63.0 b	
Tilia cordata (AMF)	38.6 a	33.5 a	
Tilia cordata (ECM)	87.7 a	91.7 a	



- Inoculated A. campestre still had higher root colonization then non-inoculated plants
- No difference in root colonization was found between +I and –I plants in linden, both for ECM and VAM



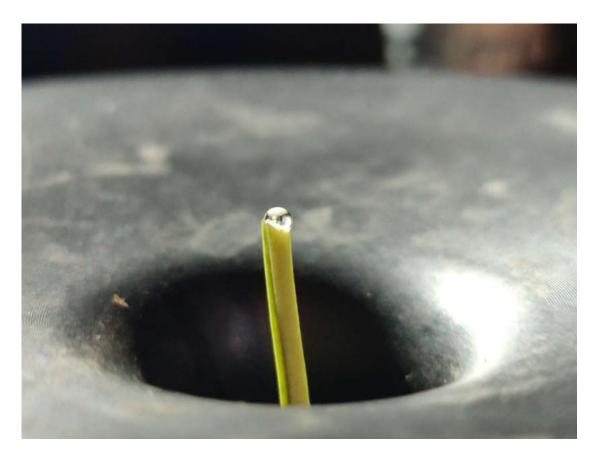
Leaf gas exchange

- In Acer, both stomatal conductance (gs) and net photosynthesis (A) were consistently increased by inoculation in the 30 months after transplanting
- In *Tilia*, gs and A were not consistently affected by inoculation
- gs is a measure of stomatal opening
- A is the net quantity of atmospheric CO₂ converted into carbohydrates by photosynthesis

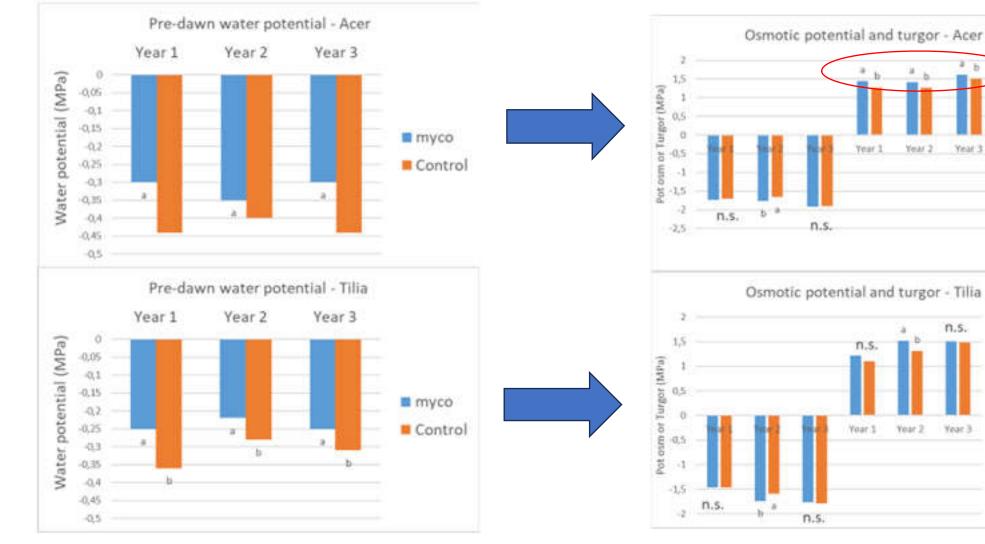
Water relations

- **Pre-dawn water potential** is major index of plant hydration.
- It is a tension (negative pressure)
- The closer to 0, the better the hydration
- It is made by two components: osmotic (negative) and turgor (positive)
- The **turgor pressure** is exerted by the plasmalemma on the cell wall, if it declines below a critical threshold, leaves wilt.
- The osmotic component is due to compatible solutes (e.g. soluble solids) within the cell. Lowering OP can help maintaining turgor at decreasing water potential (osmotic adjustment)

Water potential = Osmotic + Turgor



Water potential and its constituents



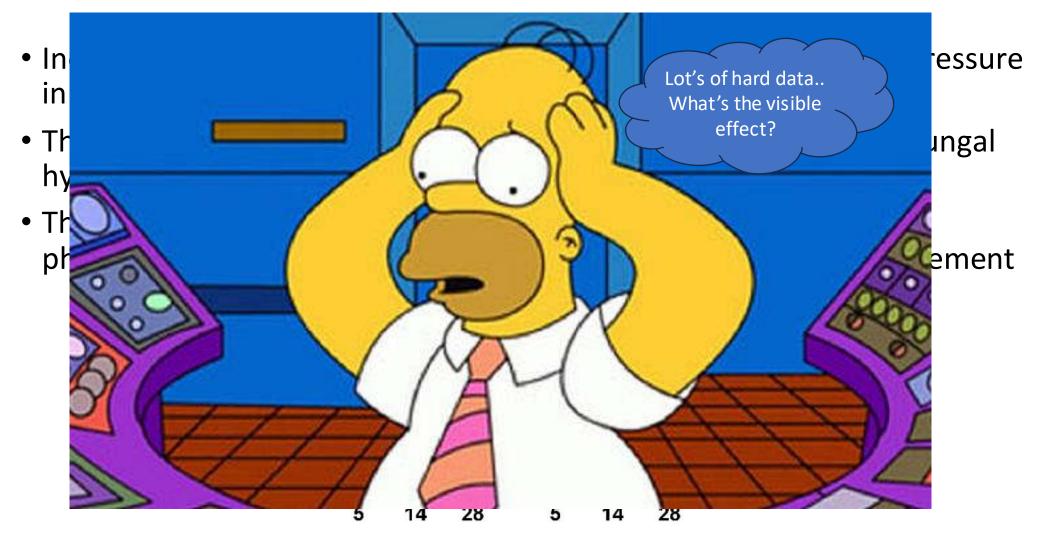
Myco

Control

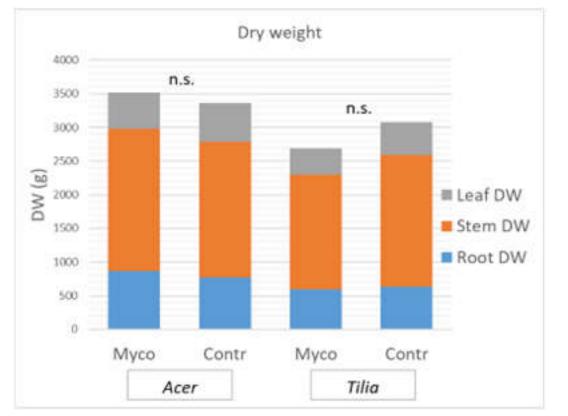
Myco

Control

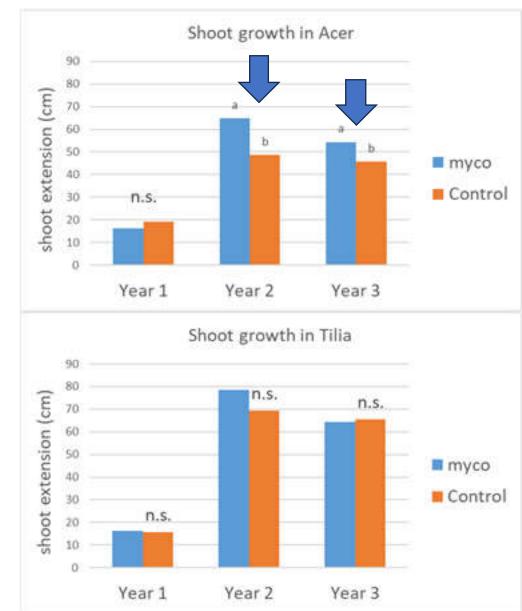
Water potential and soluble carbohydrates



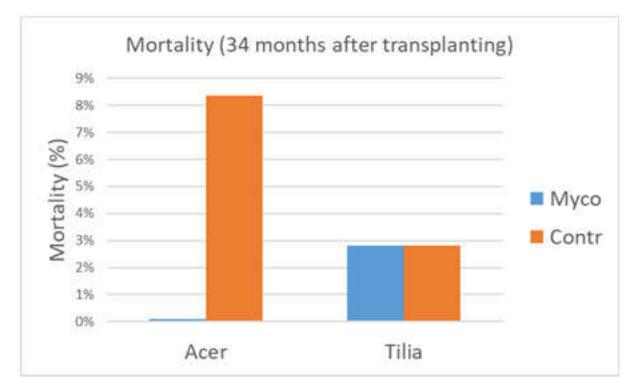
Growth after transplanting



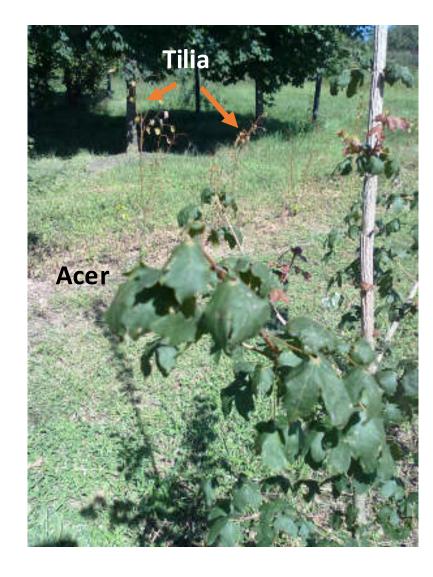
- Dry biomass on 34 MAT was not affected by inoculation
- Shoot growth increased in +I maple on year 2 and 3.



Mortality after transplanting



- Inoculated A. campestre showed no mortality after transplanting, whereas it was around 8% for noninoculated maples
- Inoculation did not improve survival in T. cordata



Mycorrhiza – take home message

- Plants inoculated in the nursery with specific symbionts sustained higher CO2 assimilation and more favorable water relations after transplanting. The effectiveness of inoculation may be lower in species such as Tilia cordata which commonly establish generalist mycorrhizal symbioses in the open field
- Controlled inoculation in the nursery is technically feasible and may be more cost-effective than inoculation at transplanting (don't use systemic fungicides!)
- Need for a better understanding of plant species—fungal strain interactions, which might be achieved by sequencing the specific fungal communities in nurseries and at urban sites.



Thanks to





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