Cultivar differences in root development and functions of grassland legumes **(RootLeg)**

Frøafgiftsfonden (2022-2023)

Frøafgiftsfonden

BELOWGROUND CROP ECOLOGY
Groundbreaking

Acknowledgment

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Frøafgiftsfonden

Root system development and function

Crop resource use efficiency

Environmental impacts

Plant growth and productivity

Climate resilience

Forage legumes are not investigated much for the **root traits**, and knowledge of cultivar differences in **root system development and function** is lacking

More knowledge on **belowground traits** is important for future breeding of resource-efficient and climate-resilient forage legumes

Photo: NR Dhamala

Objectives

- To study **cultivar differences** in root **growth and depth** over time, and root-zone activity of the three important forage legumes:

  Lucerne (*Medicago sativa* L.)

  Red clover (*Trifolium pretense* L.),

  White clover (*Trifolium repens* L.), and

- To support **future breeding** of more robust, climate and environmentally friendly forage legumes

3 Work packages (WPs)

WP1: Screening in rhizotron tubes **(Rhizotube experiment)**

WP2: Screening in the field **(minirhizotron experiment)**

WP3: Intercropping in root towers **(Root Tower experiment)**

WP1: Screening in rhizotron tubes **(Rhizotube experiment)**

Objectives

- To screen cultivars of red clover, white clover and lucerne for the differences in root depth, root architecture, and root growth over time
- To determine early root growth, the ability for the quick and early establishment

Methodology

❑ 2 m and 0.5 m tall (only in 2022), and Ø: 10 cm

Lucerne (LU)	Red clover (RC)	White clover (WC)
Cigale	Elara	Silvester
Musette	Amos	DLF 3536
Mezzo	Callisto	Brianna

Experimental setup with 0.5 m and 2 m rhizotubes tubes

Tubes at crop harvest at 118 days after transplanting (DAT) in 2022

Measurements

❑ Root depths

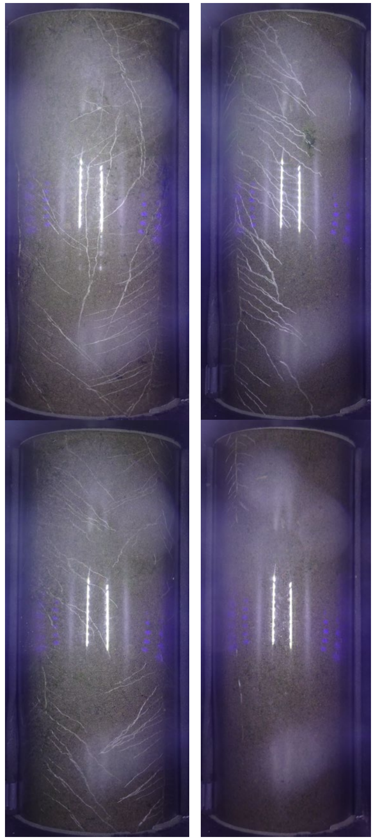
❑ Root imaging and image analysis with **RootPainter**

- Root distribution: root length density at different depths

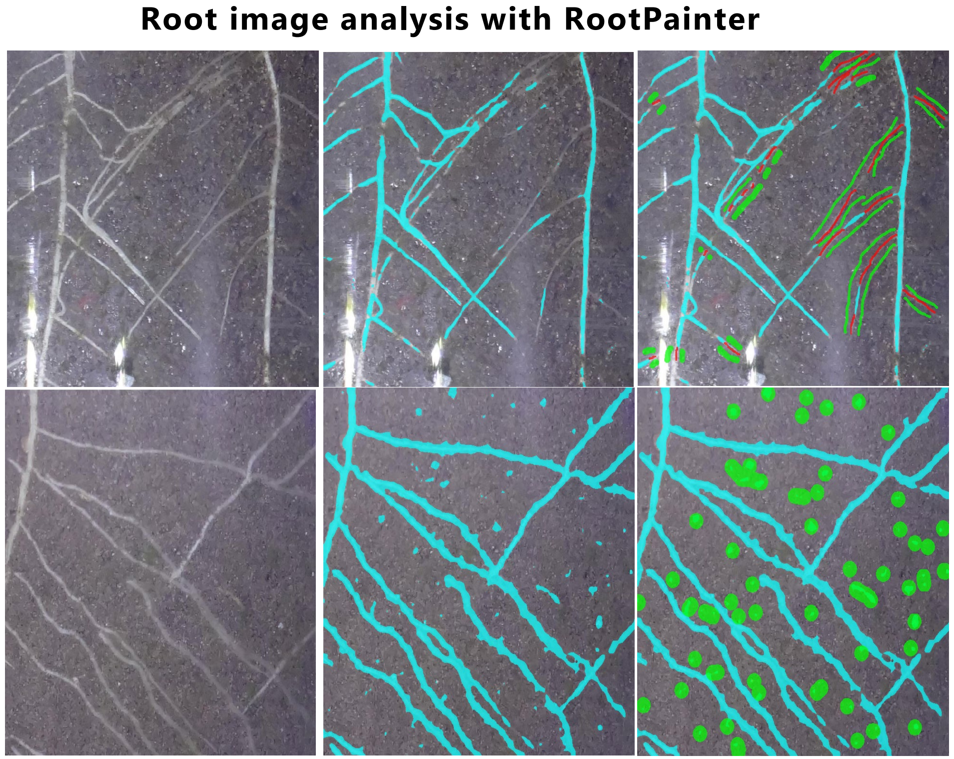
❑ Root system architecture: Visual observation and Rhizovision software

- Root biomass, total root length, and diameter

Root image analysis with RootPainter



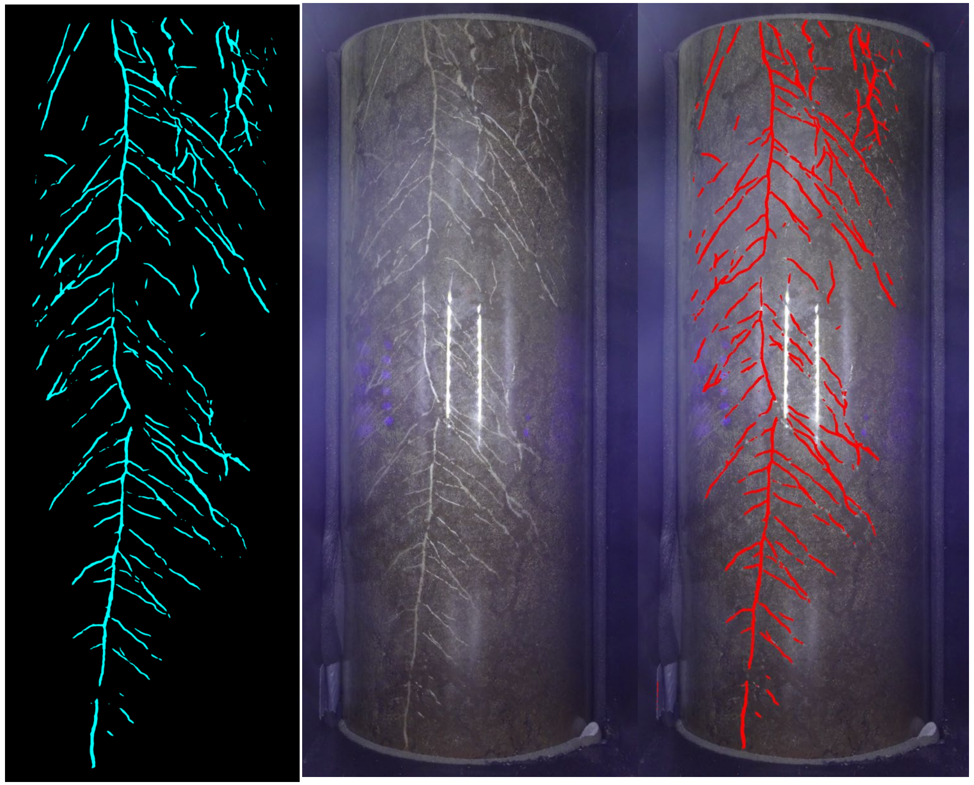
Root images



Root in tube and soil interface

AI root predictions (segmentation)

Corrective annotation



Results

Root scan analysis with RhizoVision Explorer

Root scan

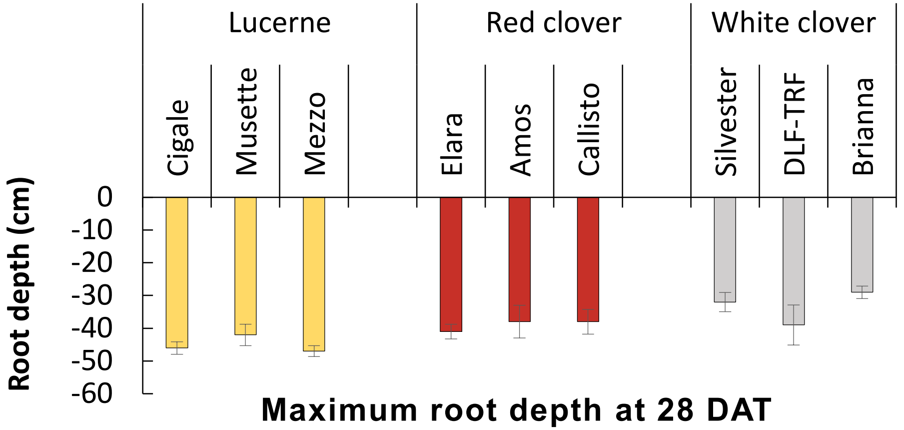
Root segmentation

Root features

Results: Rhizotube (WP1)
Root depth

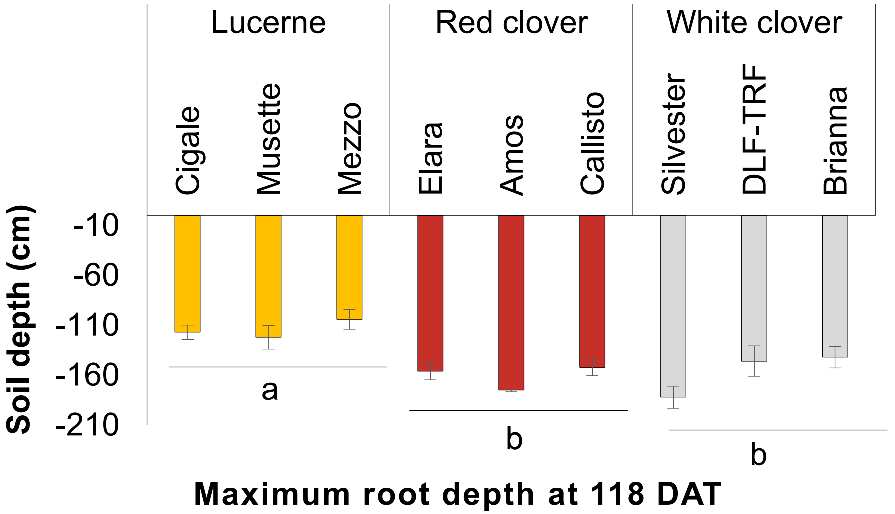
- ❑ Faster **initial root growth** of Lucerne in 50 cm tubes

(Lucerne > Red clover > White clover)



- ❑ Deeper root depth of white clover in 2022

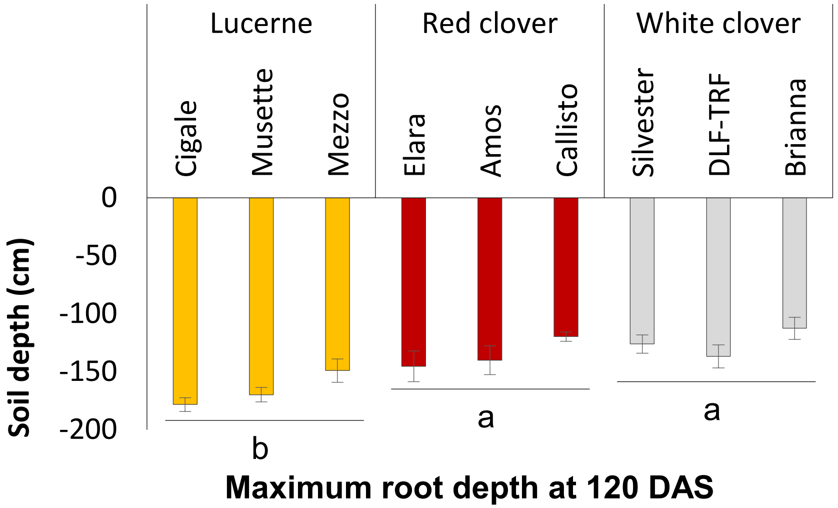
White clover = Red clover > Lucerne



The deepest root depth of the white clover **cv. Silvester** (1.82 m)

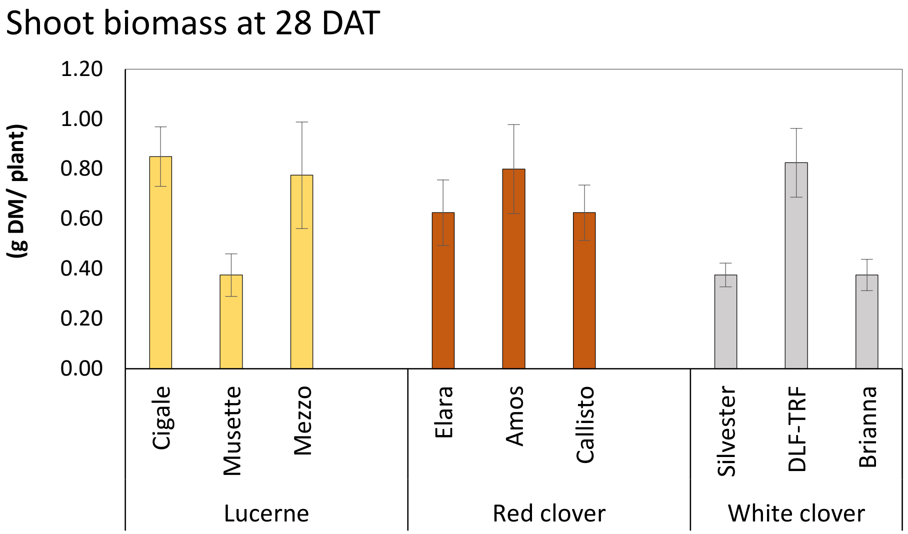
- ❑ Deeper root depth of lucerne in 2023

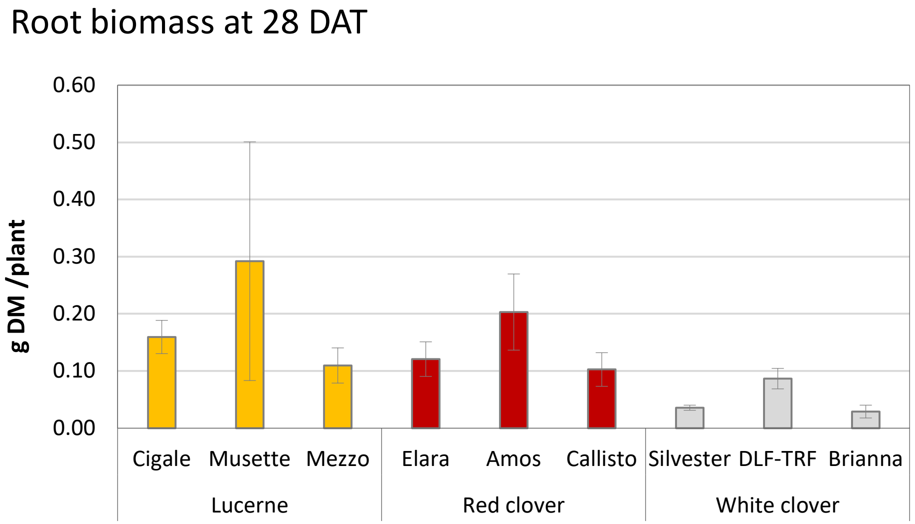
(Lucerne > Red clover = White clover)

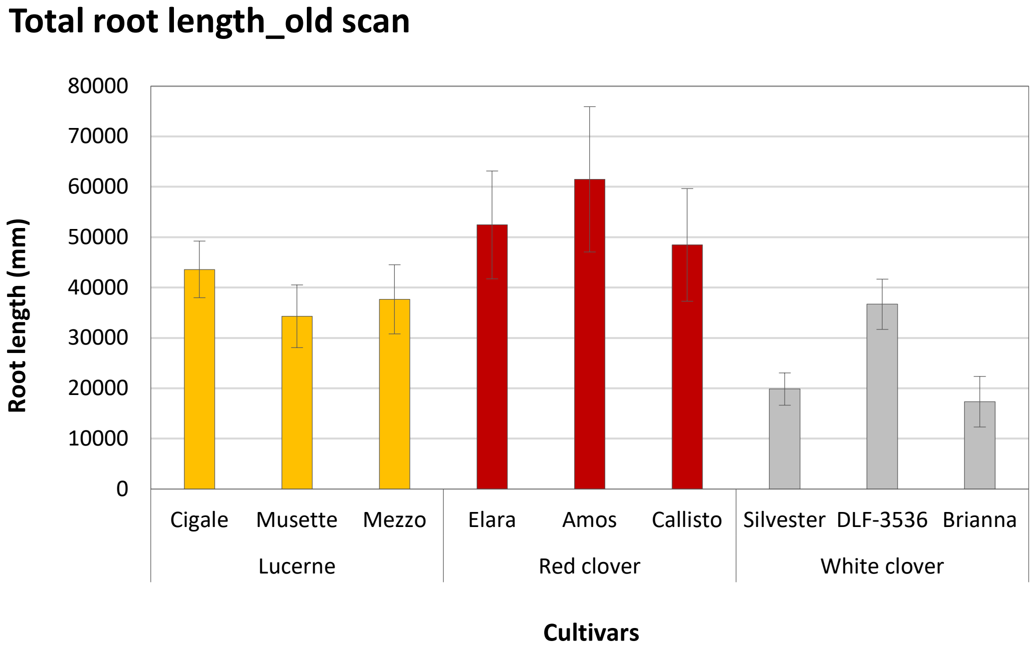


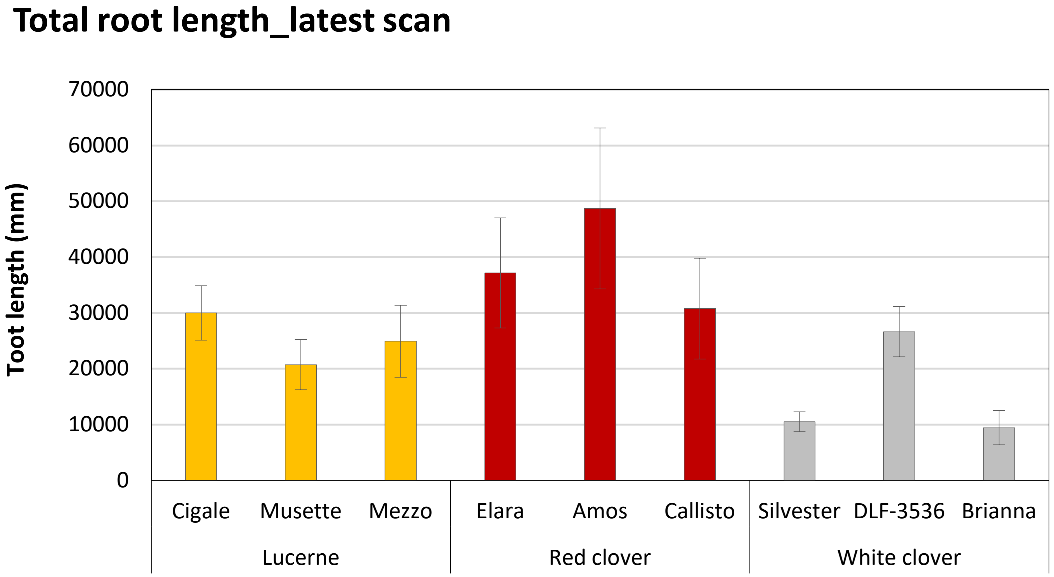
The deepest root depth of the lucerne **cv. Cigale** (1.78 m)

Root length (50 cm tube)



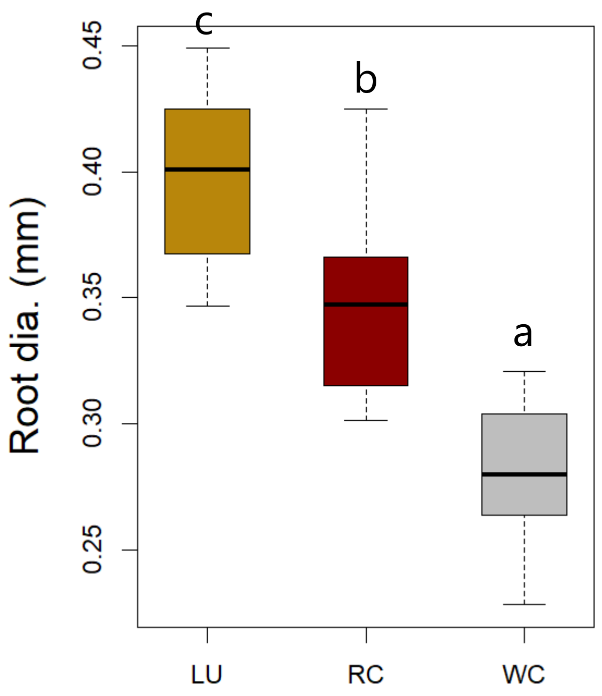


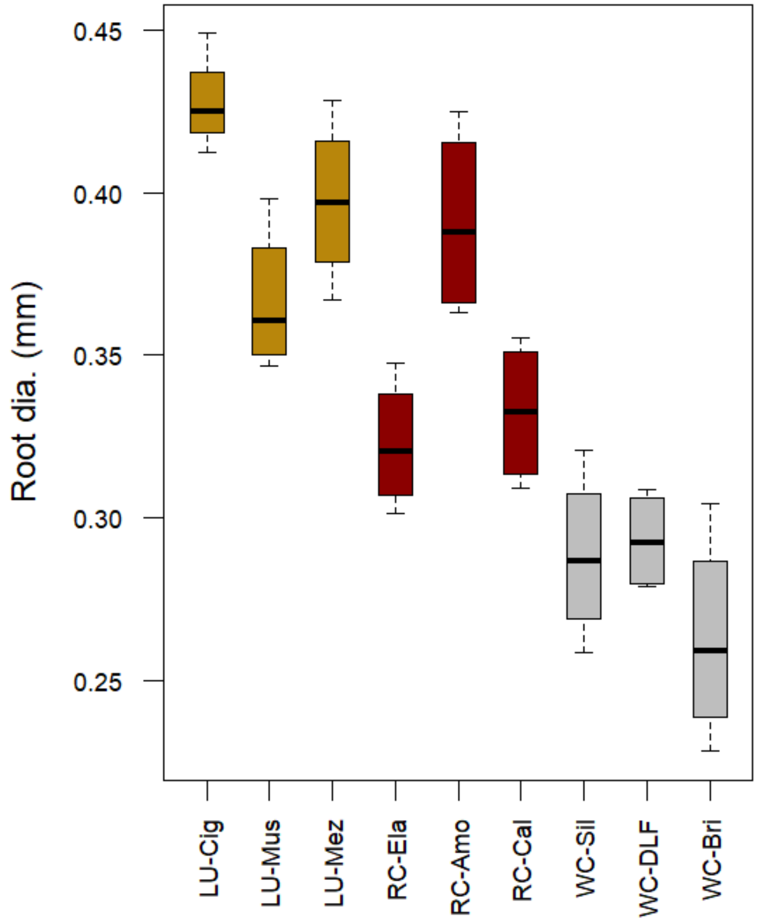


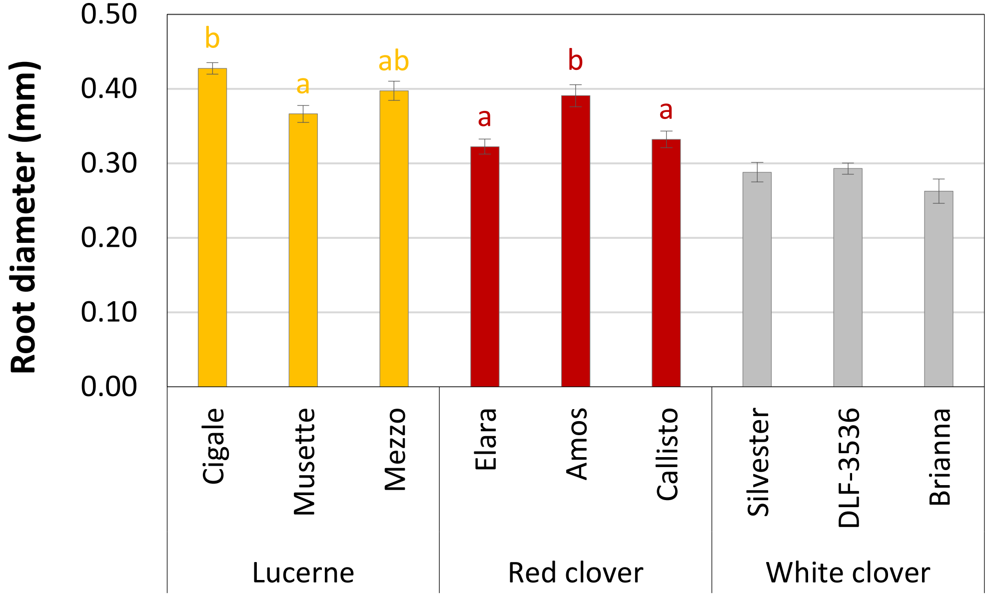


WP1 Results: Screening in rhizotron tubes (Rhizotube experiment)

Average root diameter

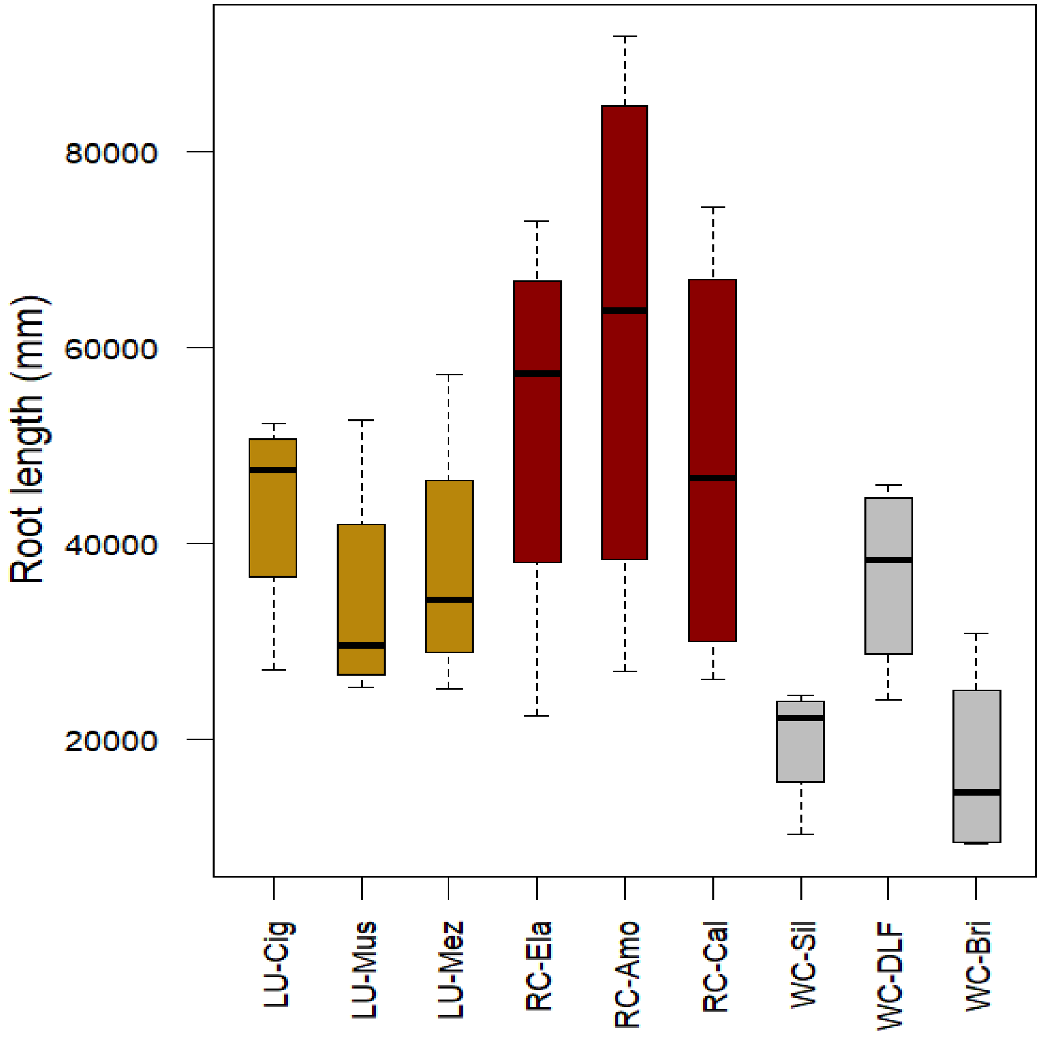




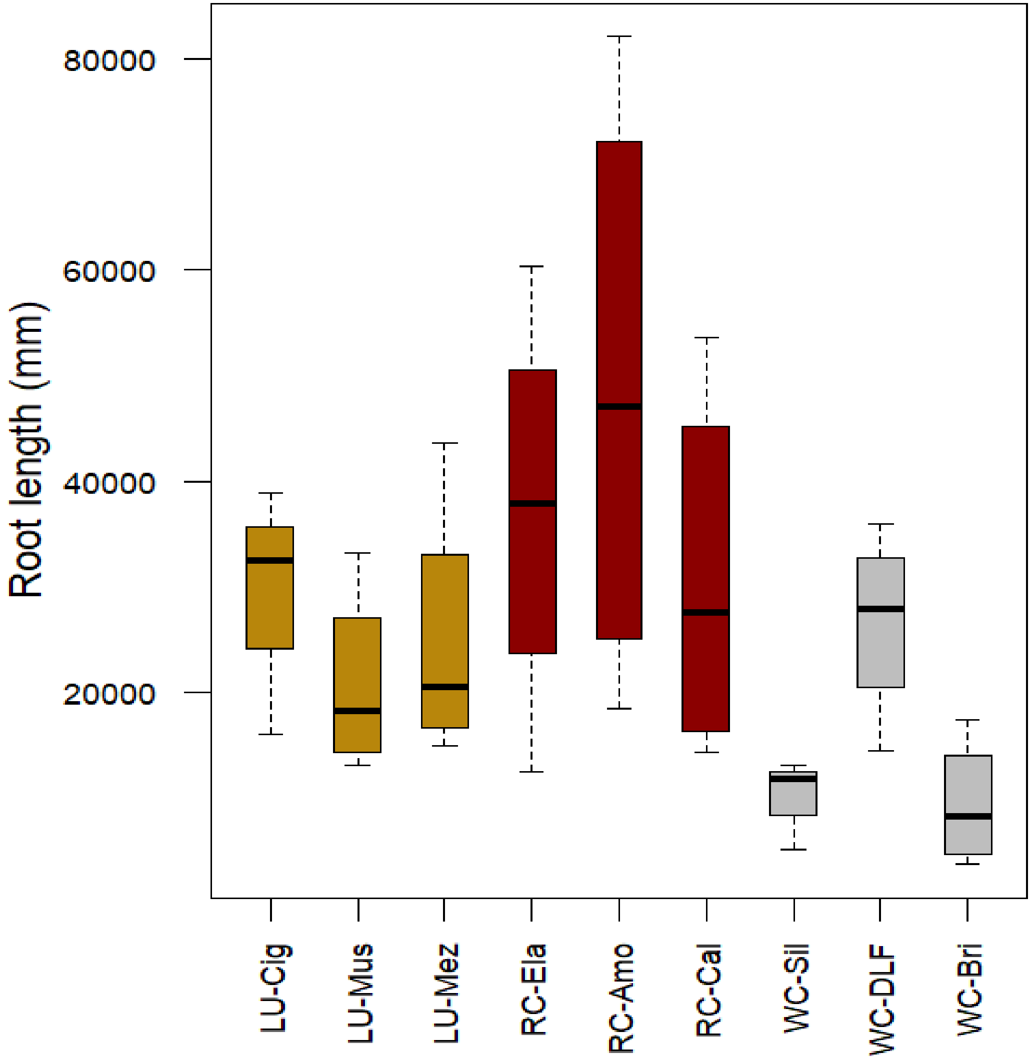


Total root length (50 cm tube)

Old scan

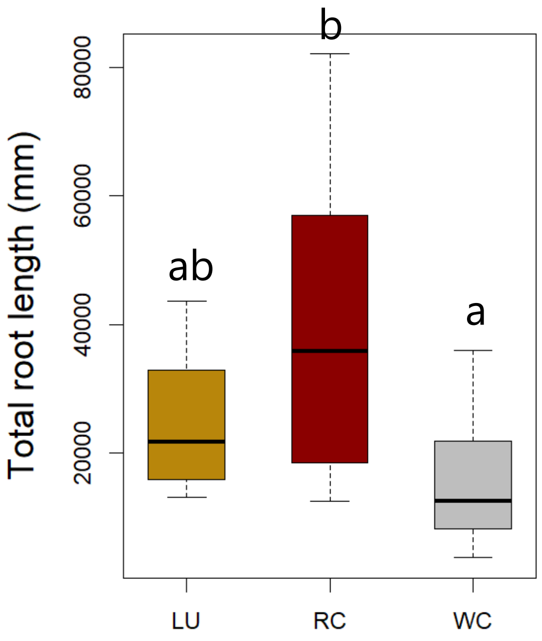


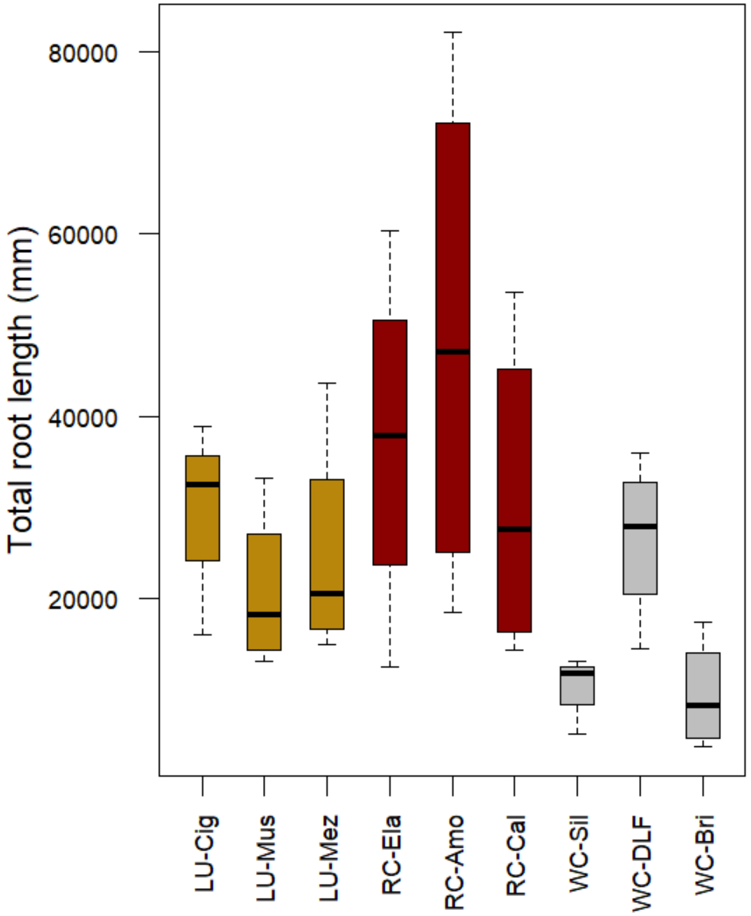
Latest scan

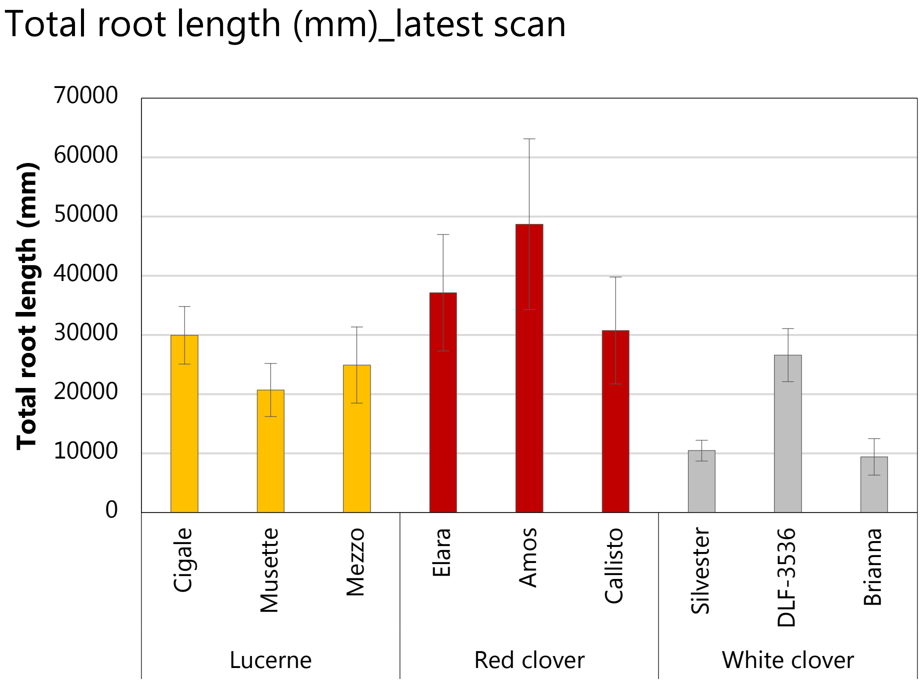


WP1 Results: Screening in rhizotron tubes **(Rhizotube experiment)**

Total root length

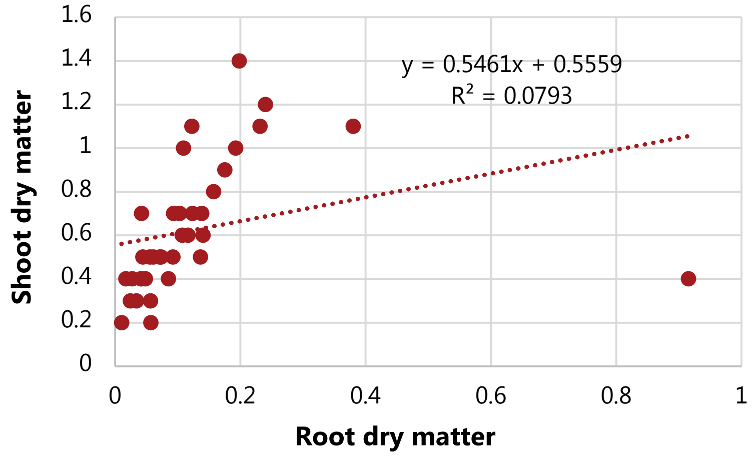


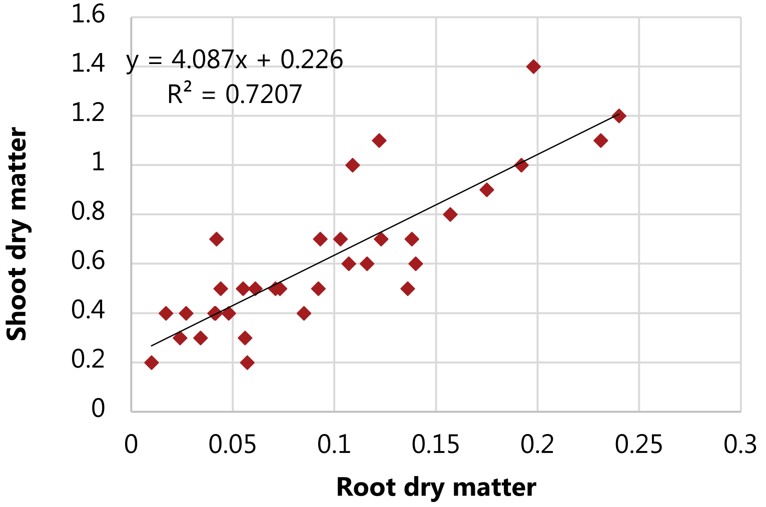




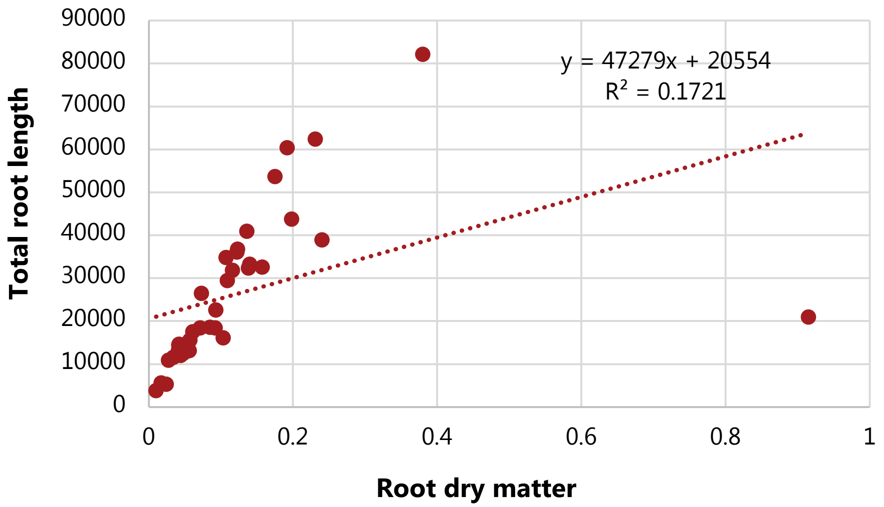
Correlations

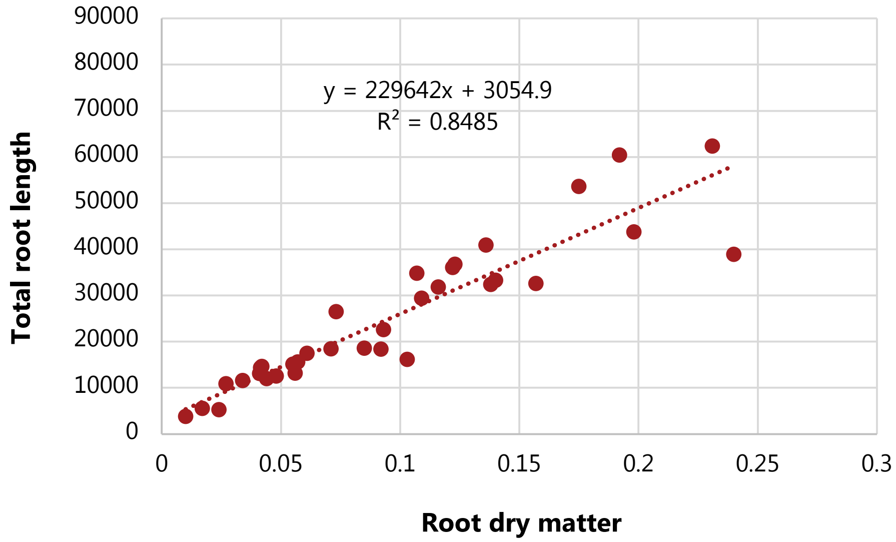
Root biomass and shoot biomass





Root biomass and total root length



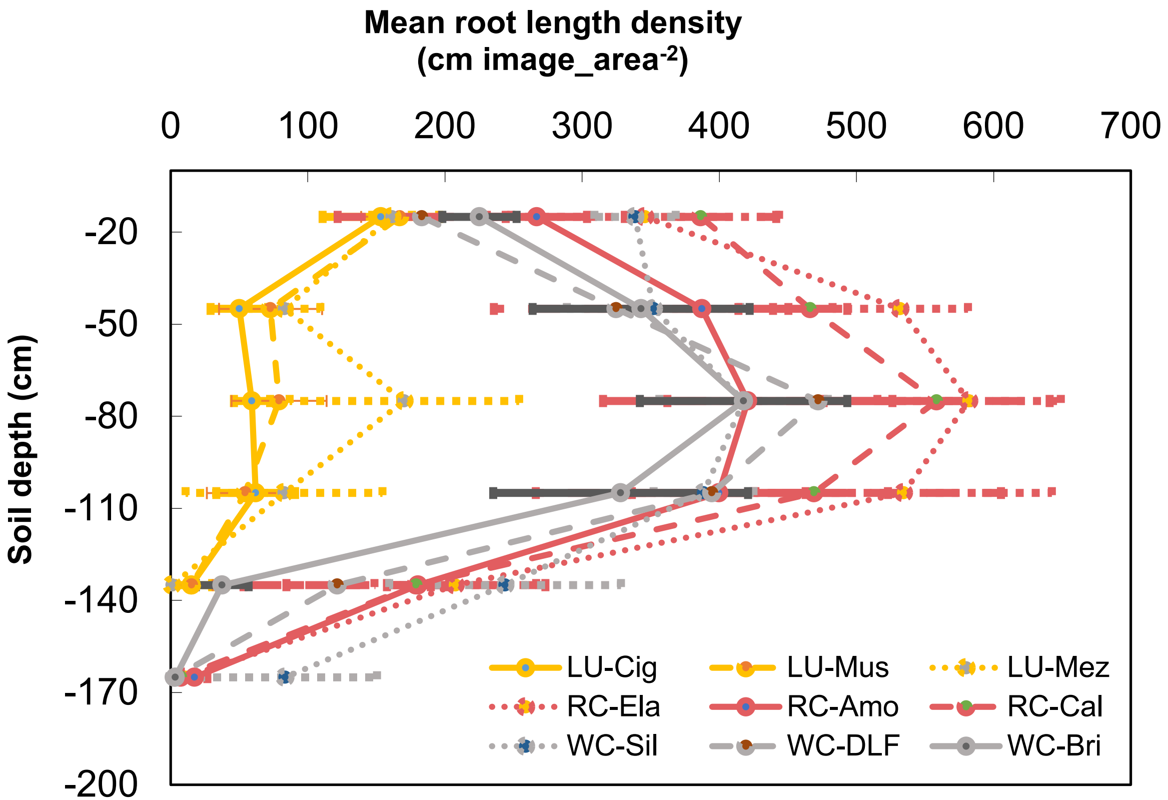


Results: Rhizotube (WP1)

Planar root length density (pRLD)

2022

Red clover = White clover > Lucerne at 0.3 to 1.2 m soil depths



(RC-Elara > RC-Cal > WC-DLF TRF)

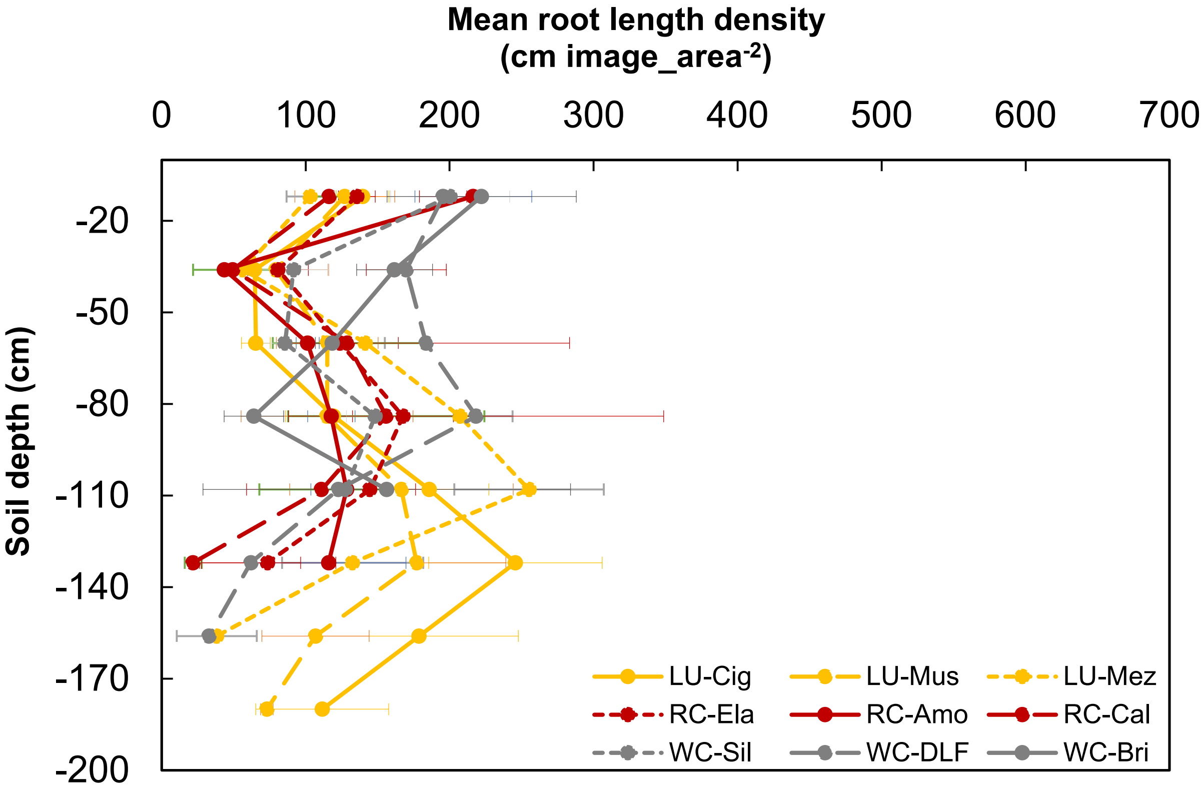
pRLD determined from the root images taken in 2 m rhizotron tubes at 118 DAT

2023

Lower pRLD compared to 2022

(White clover > Red clover = Lucerne at 0 to 50 cm soil depths

(Lucerne > Red clover = White clover below 1 m)



(Lu-Mez > LU-Cig > WC-DLF TRF > RC-Ela & Amos)

pRLD determined from the root images taken in 2 m rhizotron tubes at 120 DAS

Red clover

White clover

Roots recovered after emptying the 2m Rhizotubes at the termination of the experiment in 2022

Roots recovered and washed at 28 days after transplanting (DAT) from the 0.5 m Rhizotubes in 2022


Lucerne
cv. Cigale


Lucerne
cv. Mezzo


Lucerne
cv. Musette

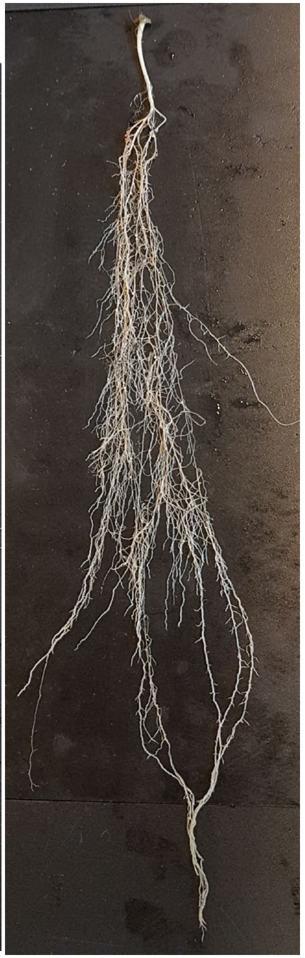

Red clover
cv. Elara


Red clover
cv. Callisto


Red clover
cv. Amos


White clover
cv. DLF-TRF


White clover
cv. Brianna


White clover
cv. Silvester

WP2: Screening in the field **(Field experiment)**

- To study cultivar differences in root growth and development of red clover, white clover and lucerne in field condition
- To develop a screening method based on **root zone activity** determined using **tracers**

Methodology

- 36 field plots (3 sp., 9 cultivars used in WP 1, with 4 replicates)
- Two 2-m deep minirhizotrons/ plot (Ø: 6 cm)

WP2: Screening in the field **(Field experiment)**

Measurements

- Seasonal root growth: root imaging every month and image analysis using RootPainter software
- Active root depth and root activity: tracer at desired depths and uptake in plant shoot biomass
- Shoot biomass production and biological nitrogen fixation

Imaging in the field

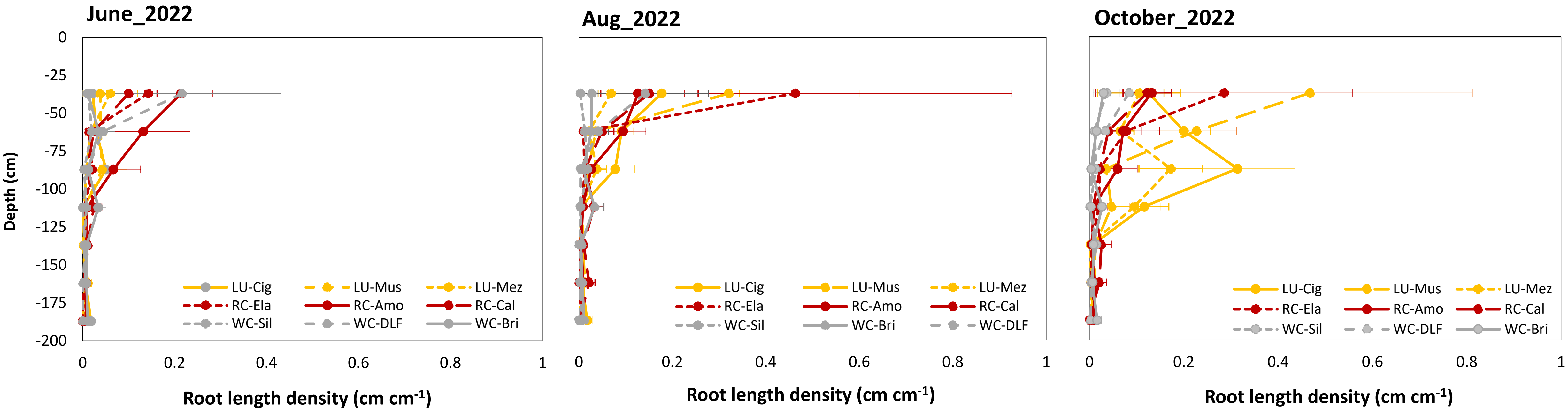
Roots in minirhizotron and soil interface

Plots injected with tracer at two different depths

Results: Field (WP2)

Planar root length density (pRLD)

2022

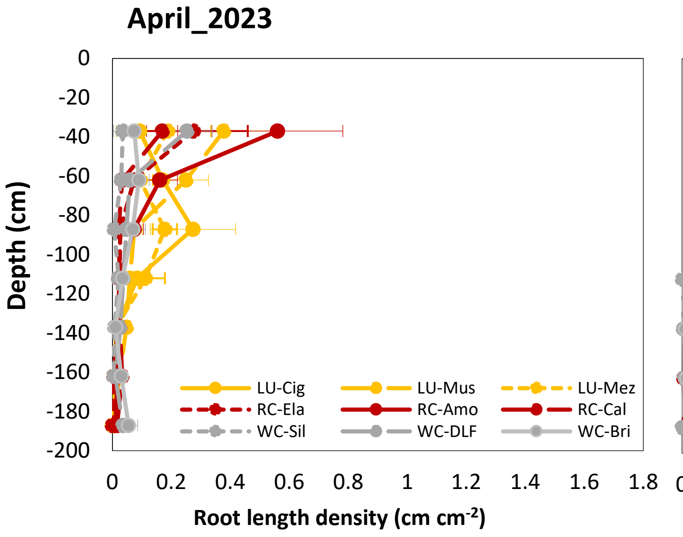


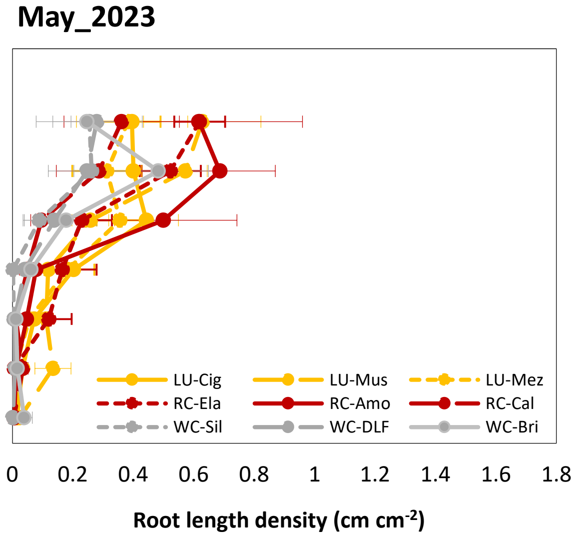
- Greater initial pRLD of red clover cultivars and WC-DLF-3536 (Red clover > Lucerne > White clover)_establishment issues
- Higher and faster increases in pRLD of LU-Mus and RC_Ela overtime during the growing season (on top soil layer)
- Higher pRLD of lucerne below 50 cm depth (Lucerne > Red clover > White clover)
- RLD of LU-Mus decrease below 50 cm depths, whereas it was increased in upto 100 cm depth

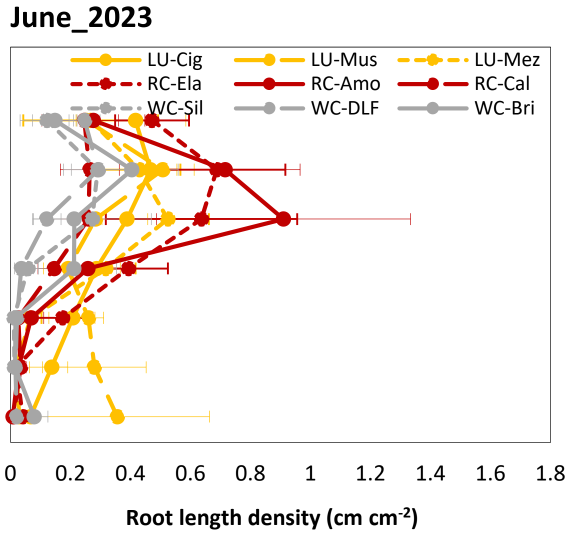
Results: Field (WP2)

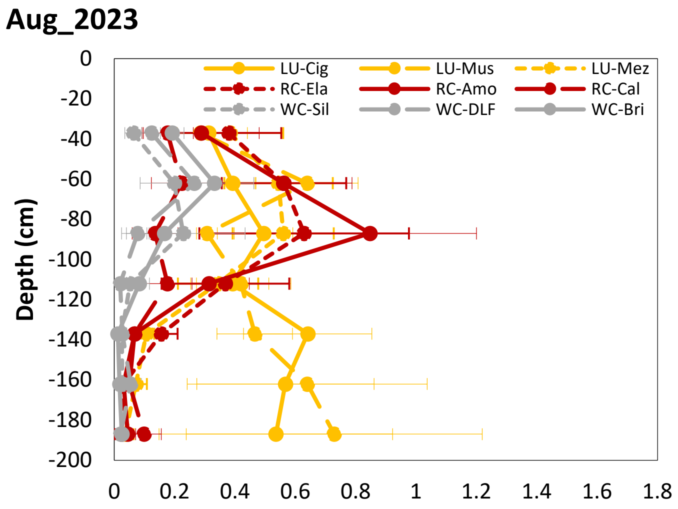
Planar root length density (pRLD)

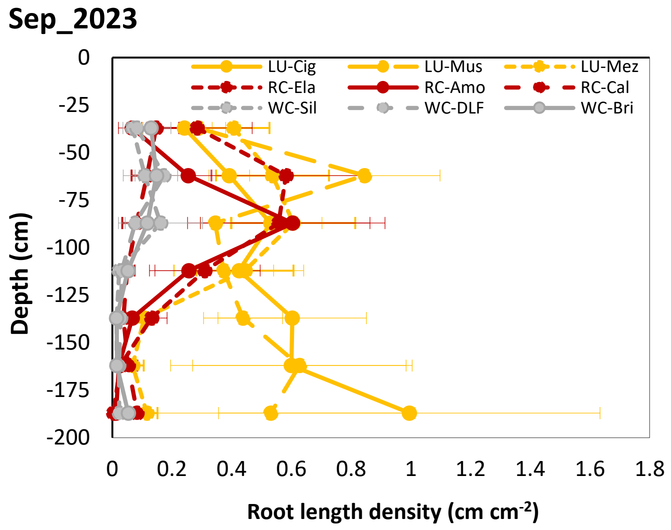
2023











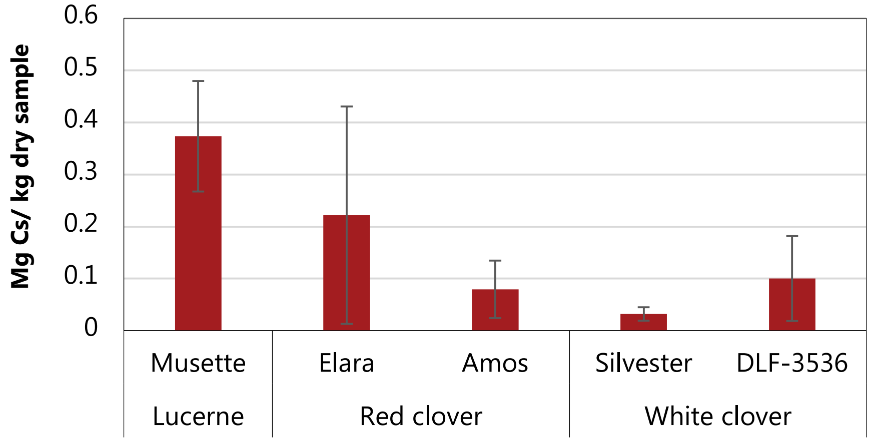
- pRLD increased in the second year (2023) at all depths
- Greater pRLD of red clover cultivars: Elara and Amos, up to 1m soil depth until Aug
- pLER of lucerne culivars: Cigale and Musette increased across deeper soil layers (below 1m) later in the growing season, but it was decreased for Mezzo
- Larger difference between the RC cultivars (Amos & Elara> Callisto) up to 1 m depth, and the lucerne cultivars (Cigale & Musette > Mezzo) below 1 m depth
- Initially, Brianna had great RLD but did not differ between the cultivars later in the growing season

Results: Field (WP2)

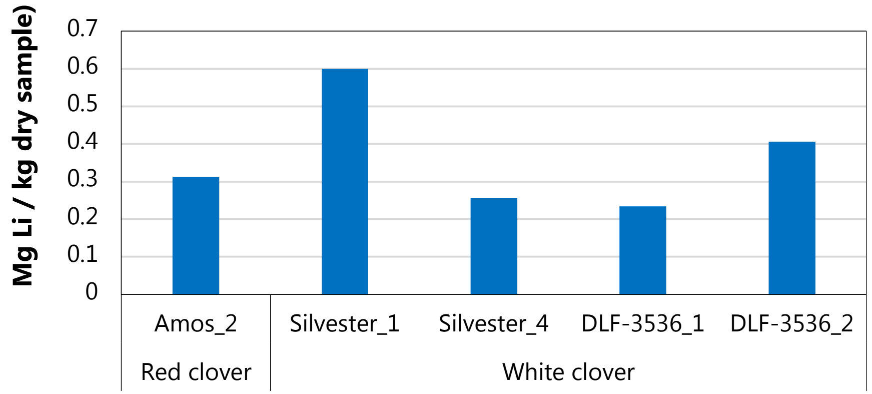
Tracer application

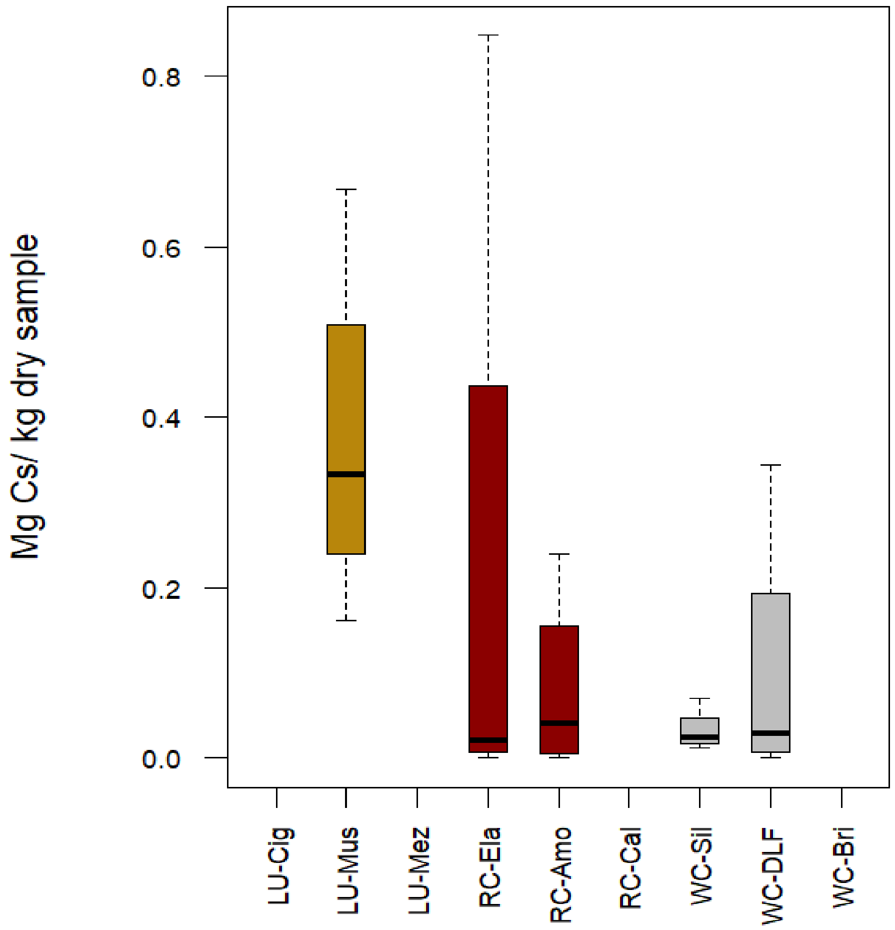
Caesium at 120 cm depth and Lithium at 80 cm depth

Caesium (Cs) enrichment



Post tracer Li concentration

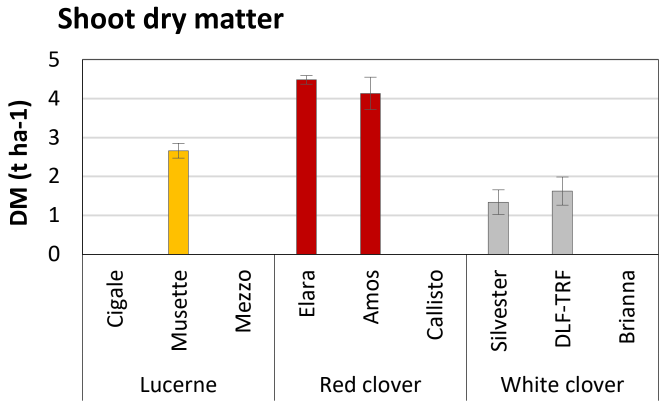


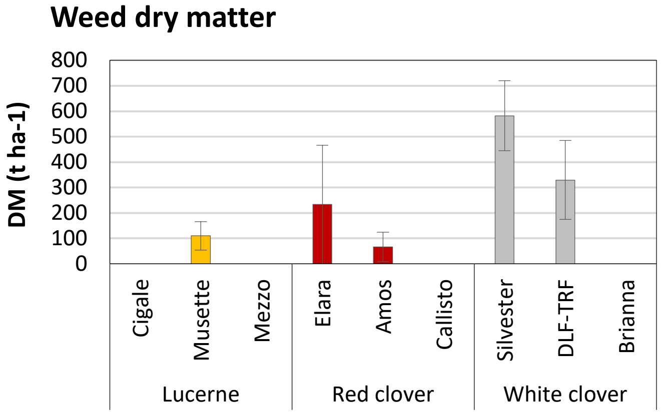


Results: Field (WP2)

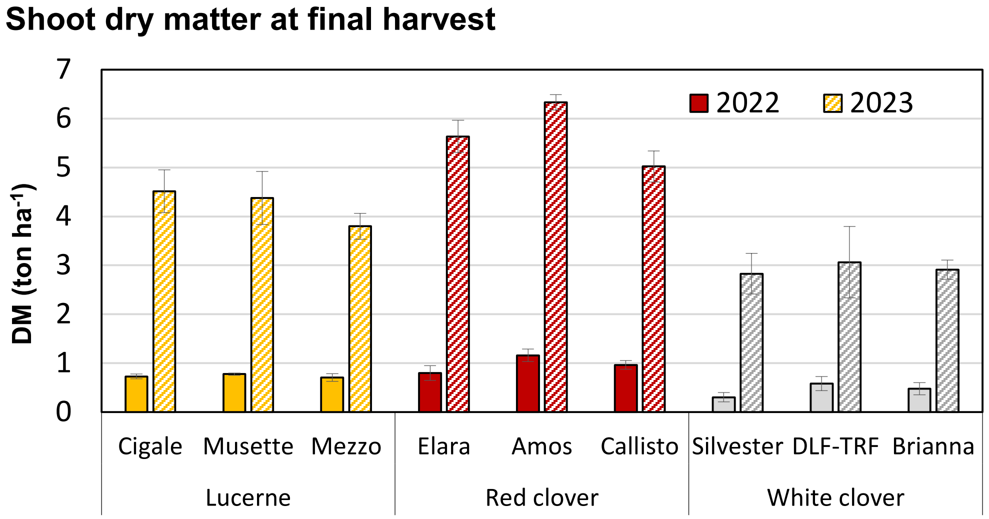
Plant production

Estimated in sub-plots (2x0.25 m^2) in 30 May, 2023

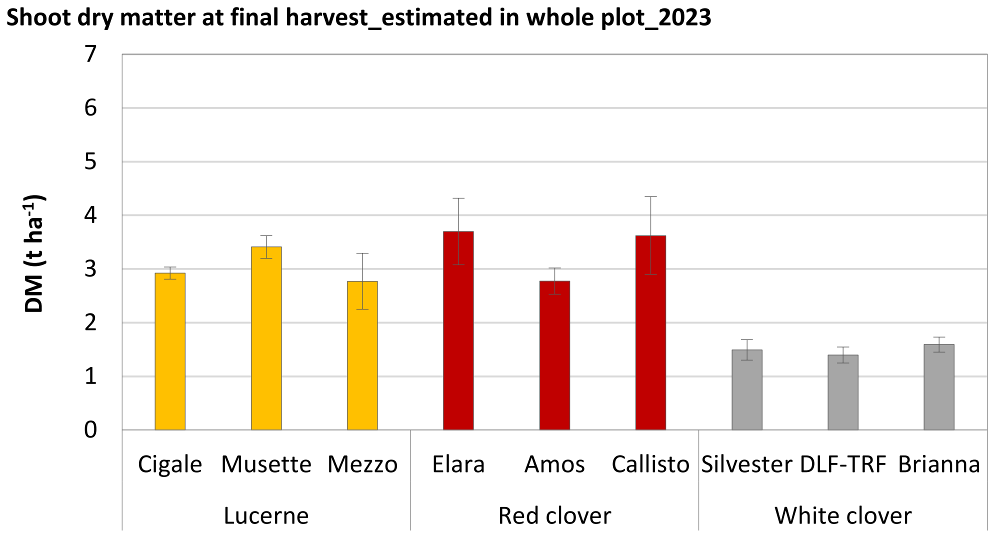


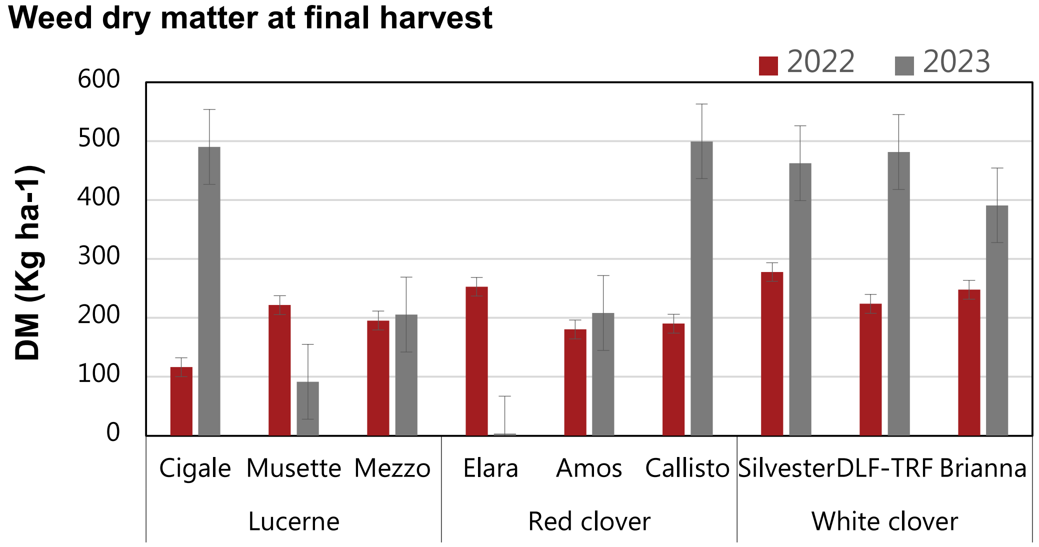


Estimated in sub-plots (2x0.25 m^2) at final harvest



Estimated in whole plot (8x1.5 m^2) at final harvest

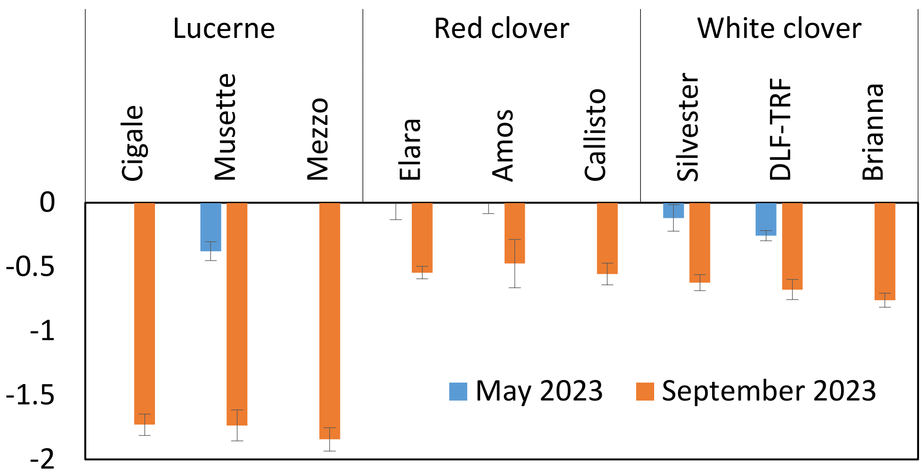




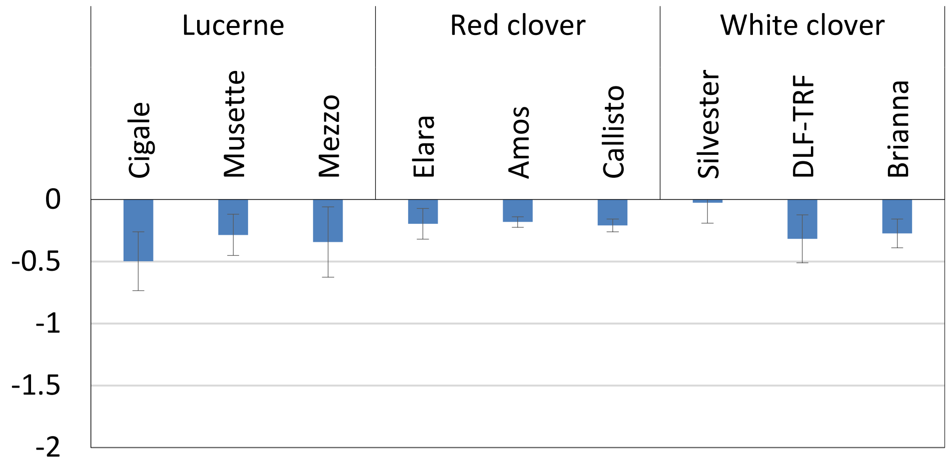
Results: Field (WP2)

N2 fixation estimation

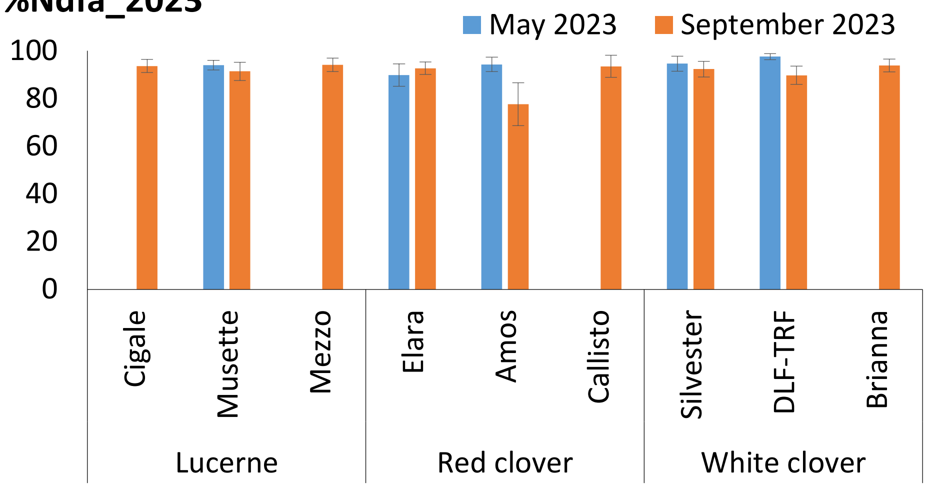
δ15N_2023



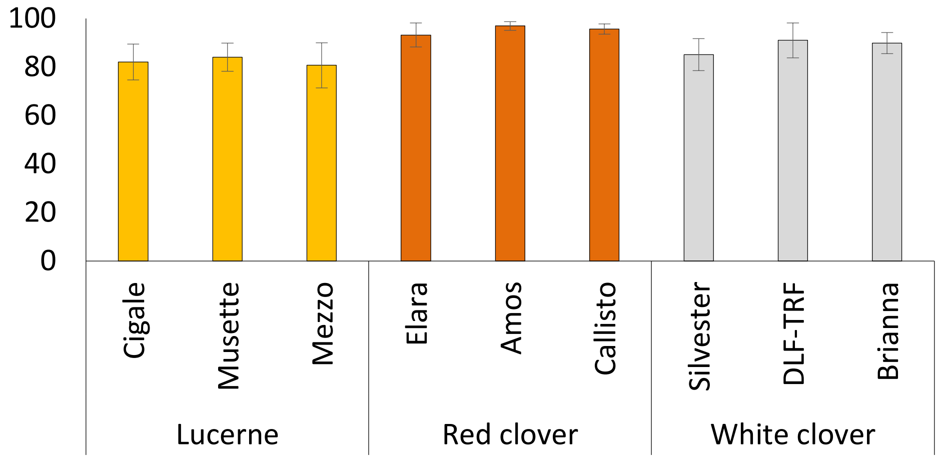
δ15N_Sep 2022



%Ndfa_2023



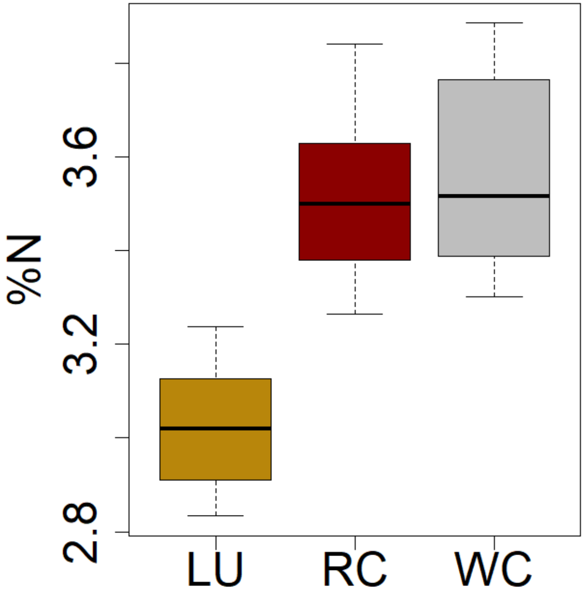
%Ndfa, Sep 2022

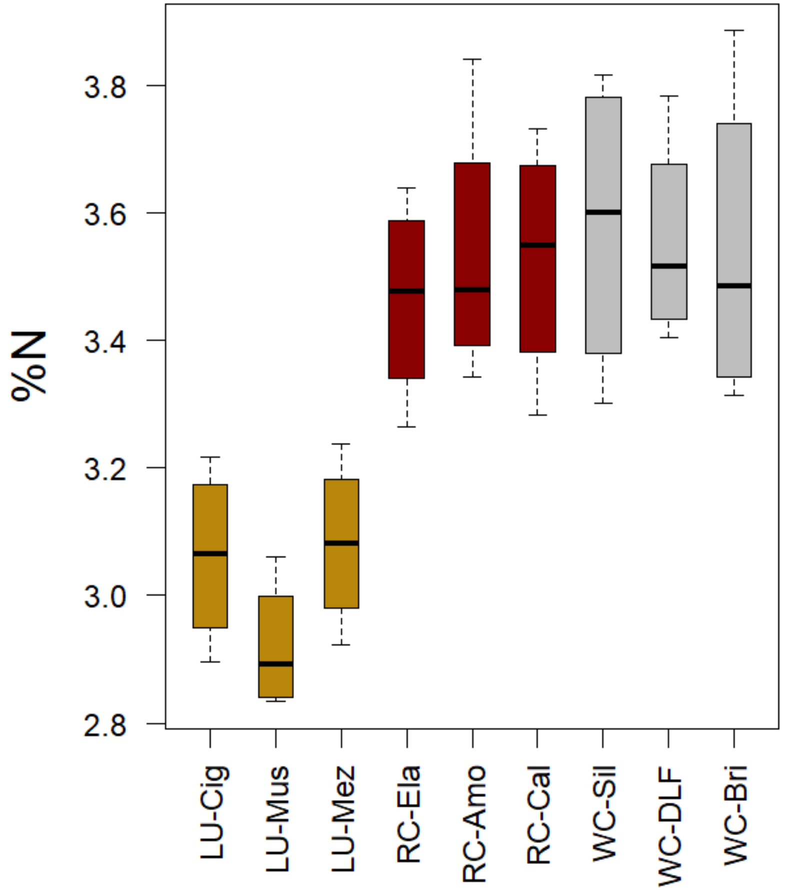


%Ndfa: Percentage of N derived from atmospheric N2-fixation estimation

N studies

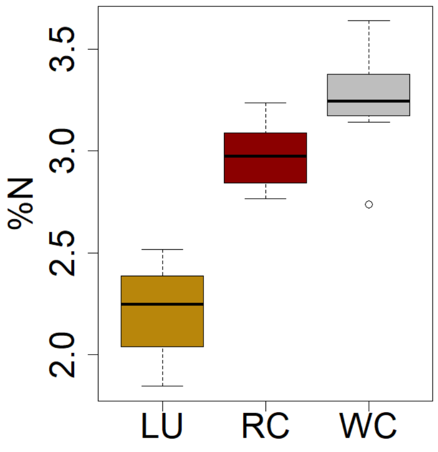
N content_2022

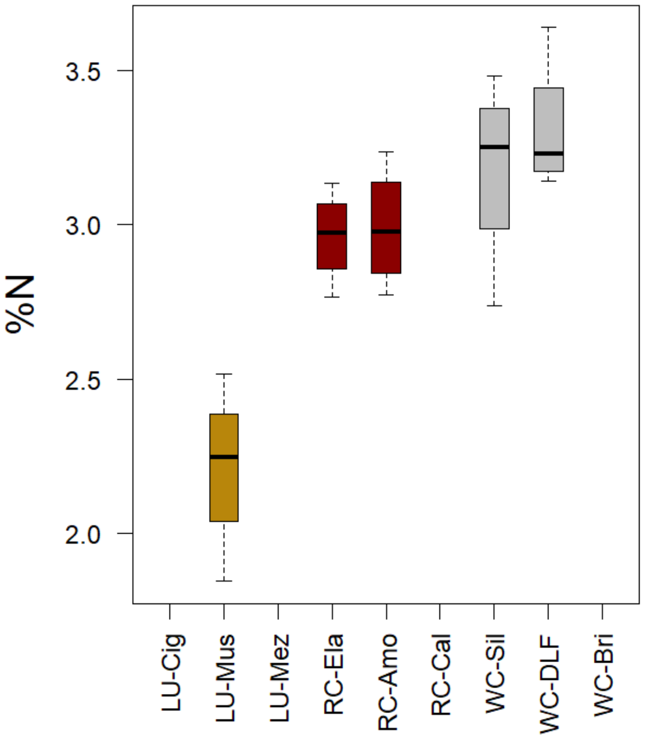




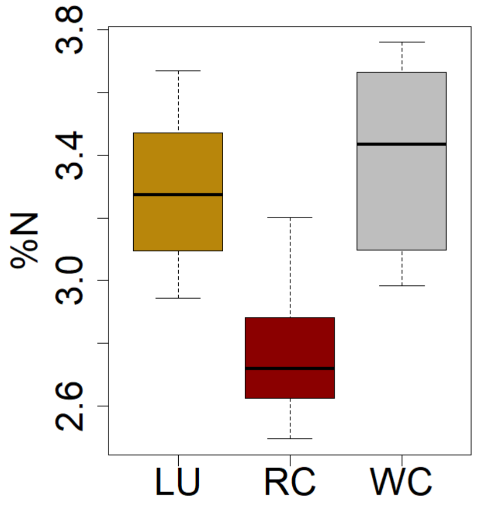
N studies

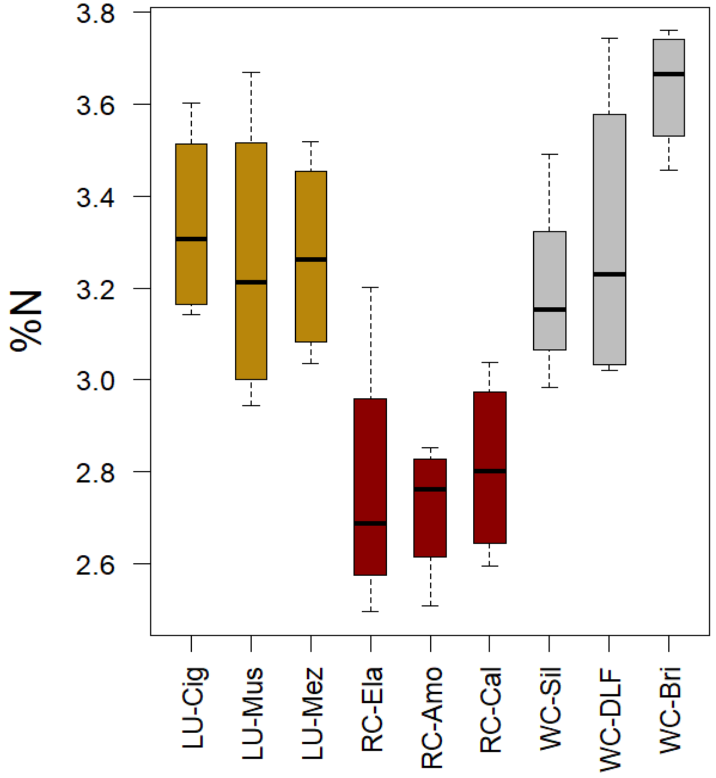
N content_2023_May





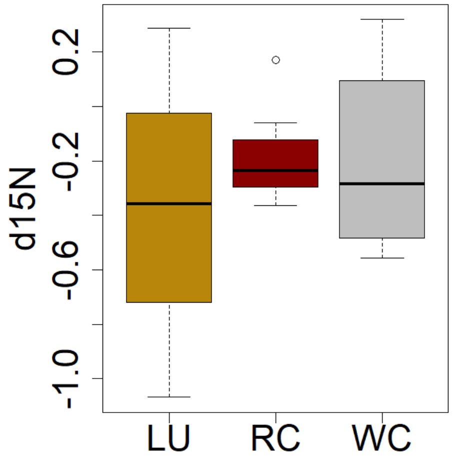
N content_2023_Sep

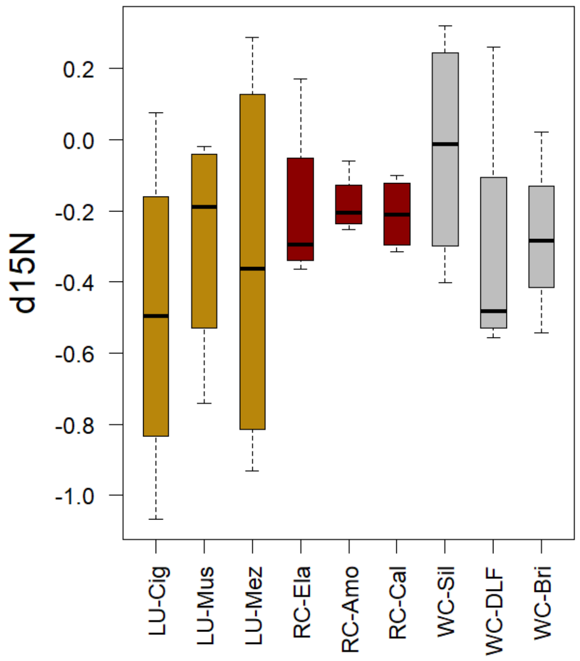




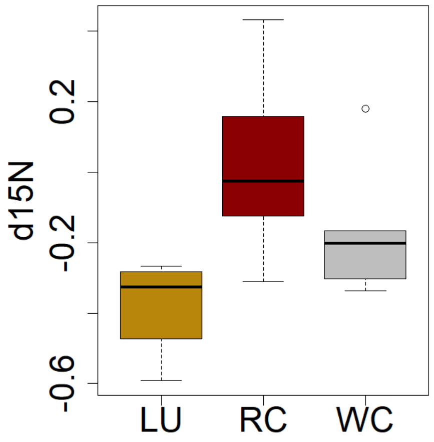
N studies

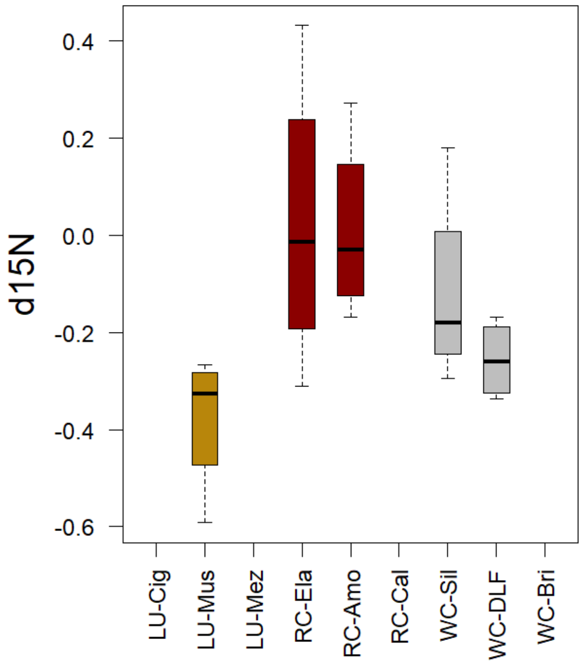
δ^{15}N_2022



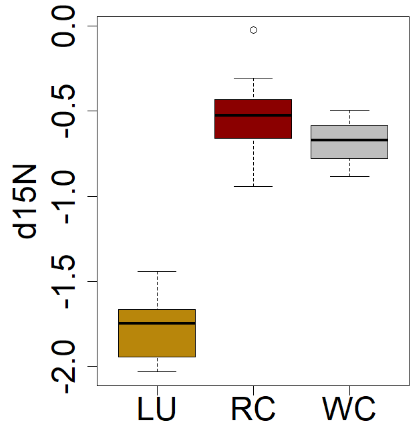


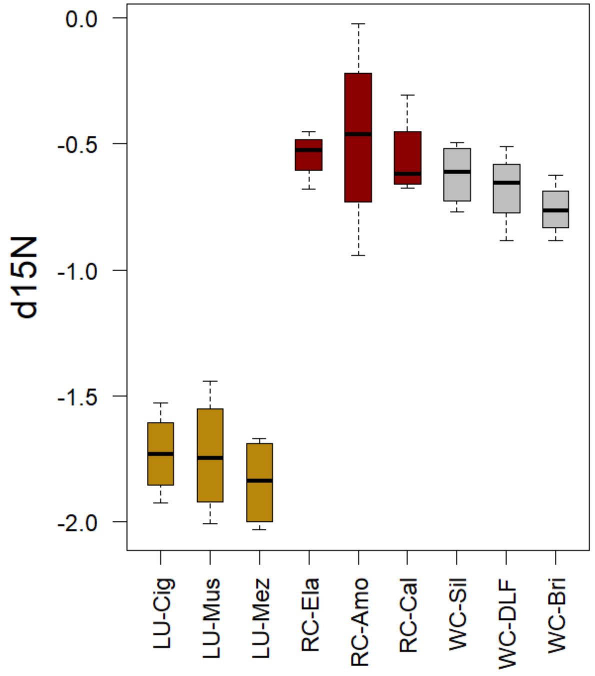
δ^{15}N_2023_May





δ^{15}N_2023_Sep





WP3: Intercropping (IC) in root towers **(Root Tower experiment)**

Objectives

- To study the effect of IC with grasses on the root development and resource uptake in grassland legumes
- To determine how it is affected when intercropped with deep or shallow rooted grass

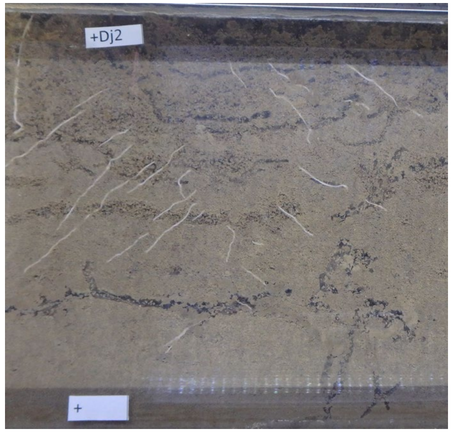
Methodology

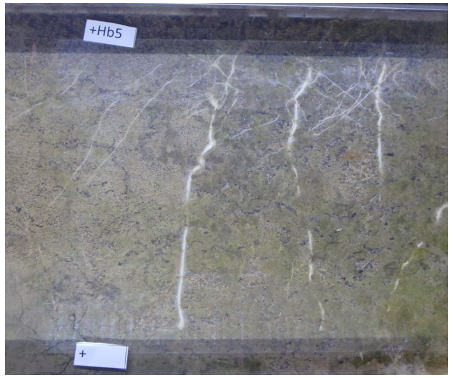
- 4m x 1.2m x 0.6m root towers
- Red clover (RC) (cv. Callisto) grown in sole crop and intercropping (IC) with perennial ryegrass (PR) and Tall fescue (TF)
- 6 crop mix treatments:
  Sole crops: Red and white clover, perennial ryegrass and tall fescu
  Intercrops: red clover+P. ryegrass & red clover+tall fescue

WP3: Root tower experiment

Measurements

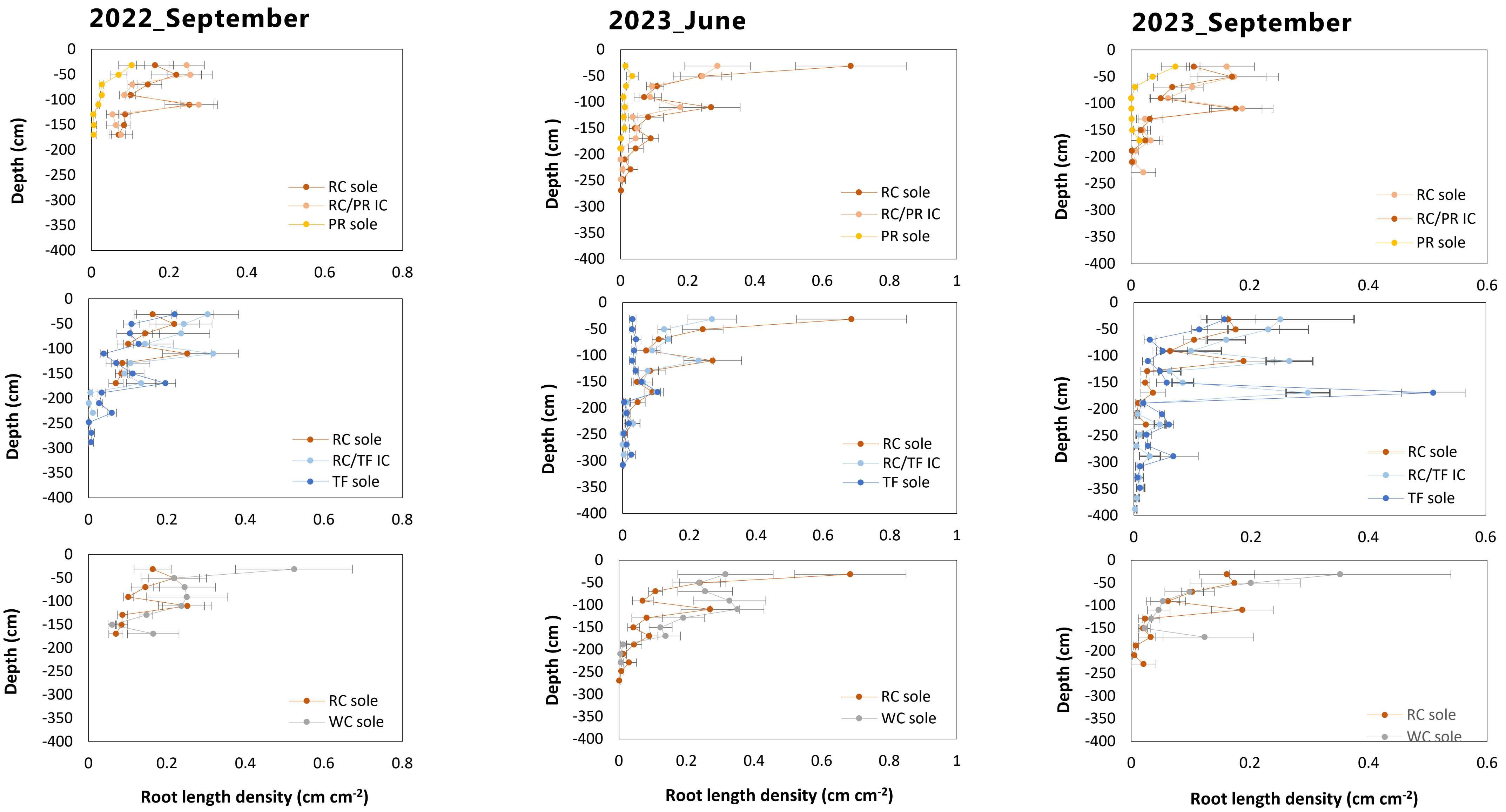
- Seasonal root growth: imaging and image analysis
- Root activity at specific depths using tracers
- Estimation of water uptake from water sensor data





WP3: Intercropping (IC) in root towers (Root Tower experiment)

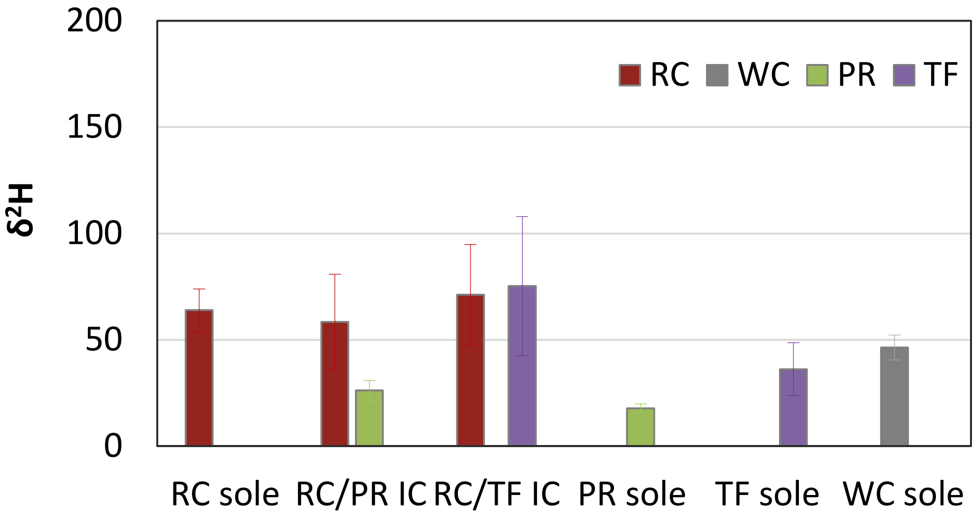
Plannar root length density (pRLD)



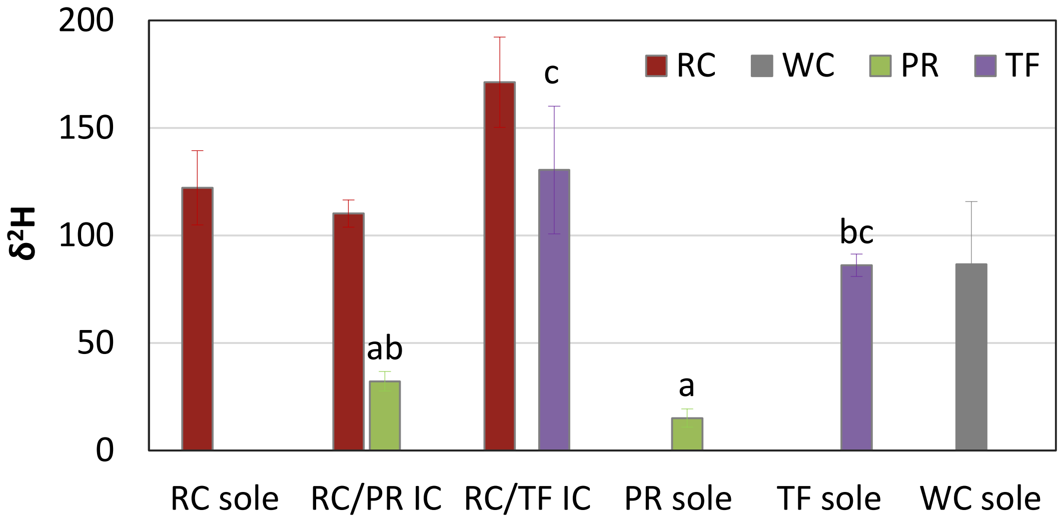
WP3: Intercropping (IC) in root towers **(Root Tower experiment)**

Uptake of ^2H enriched water (deuterium tracer) at 1.7 m depth

δ^{2}H enrichment_2022



δ^{2}H enrichment_2023



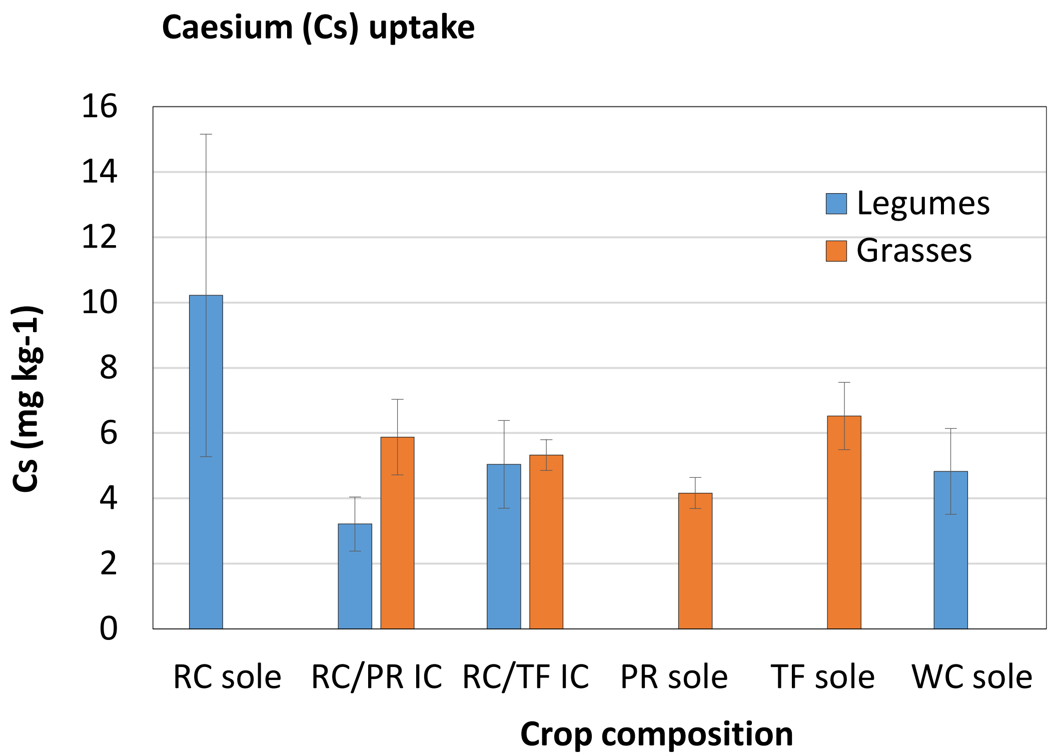
RC: red clover; **WC:** white clover; **PR:** Perennial ryegrass; **TF:** Tall fescue

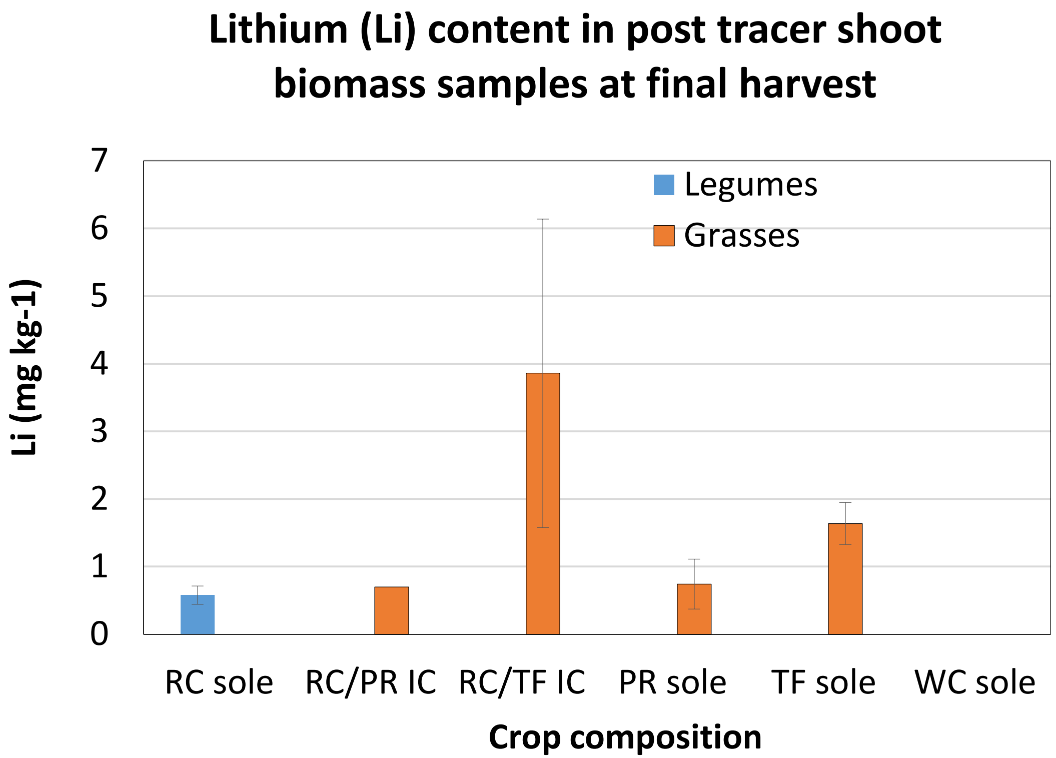
δ2H enrichment = Difference in δ2H in post tracer shoot biomass – pre-tracer shoot biomass

Results: Root tower experiment (WP3)

Tracer application:

Caesium was applied at 0.5 m and and Lithium at 2.3 m in 2023



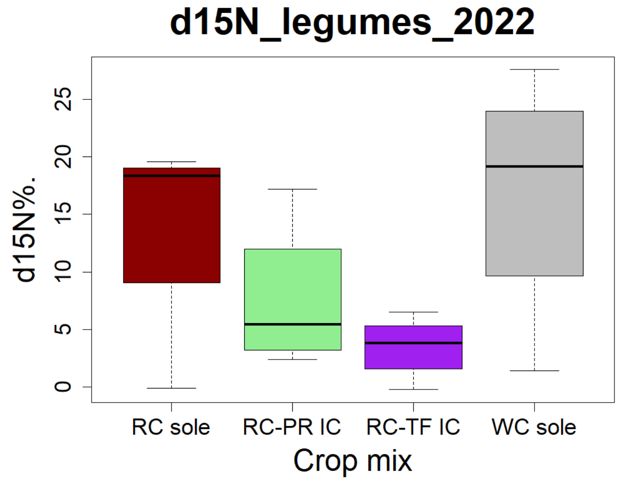


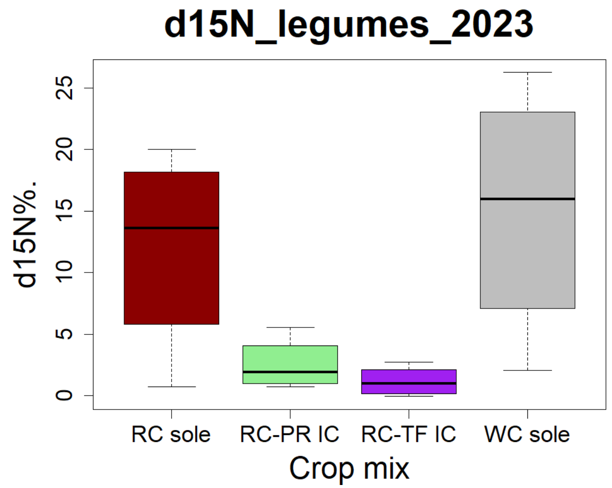
- **Caesium (Cs) uptake/ enrichment = Difference in Cs contect in post tracer shoot biomass at final harvest - Cs in pre-tracer shoot biomass**
- **Lithium (Li) content was below measurement limit in the pre- tracer shoot biomass**

RC: red clover; **WC:** white clover; **PR:** Perennial ryegrass; **TF:** Tall fescue; **IC:** intercropping

WP3: Intercropping (IC) in root towers **(Root Tower experiment)** $d^{15}N$ of legumes

$\delta^{15}N$ of legumes

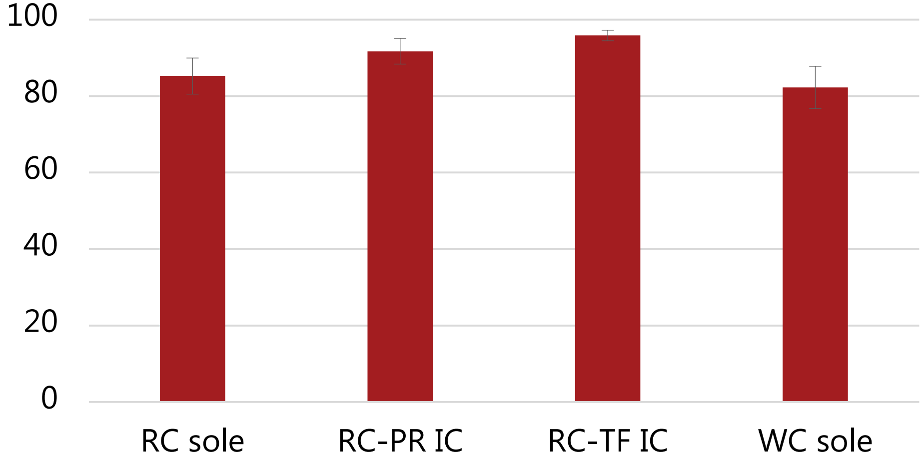




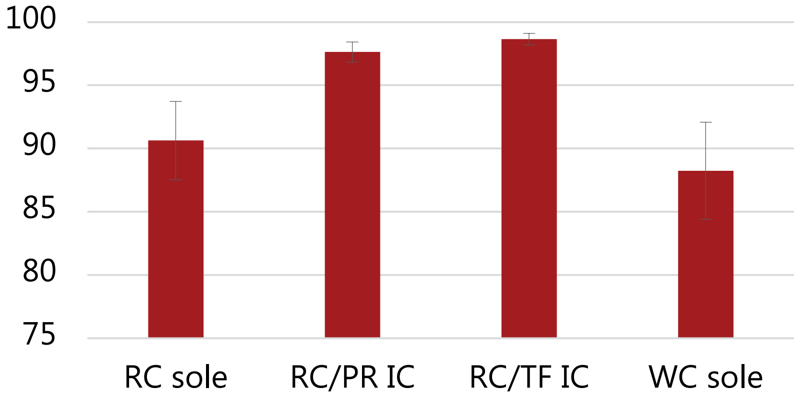
RC: red clover;
WC: white clover;
PR: Perennial ryegrass;
TF: Tall fescue
IC: Intercropping

Red clover %Ndfa

2022



2023

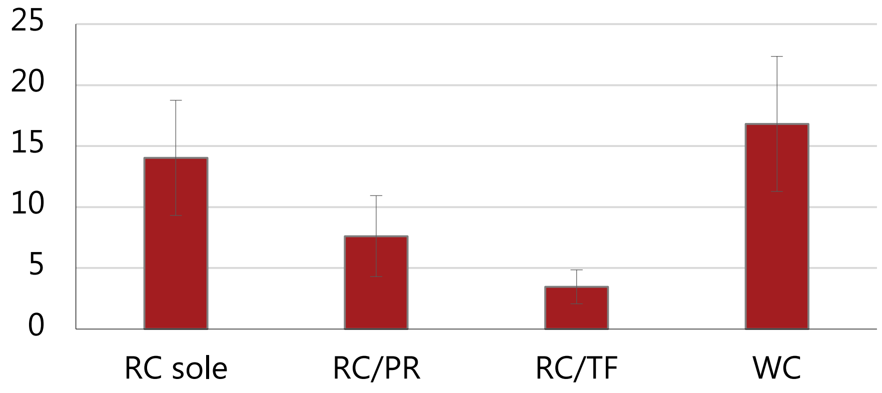


%Ndfa: Percentage of N derived from the atmospheric N_2 fixation

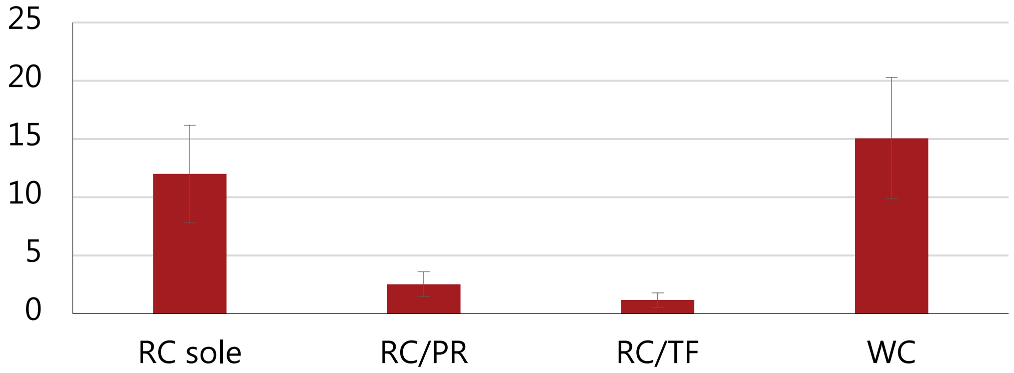
Results: Root tower experiment (WP3)

N2 fixation estimation

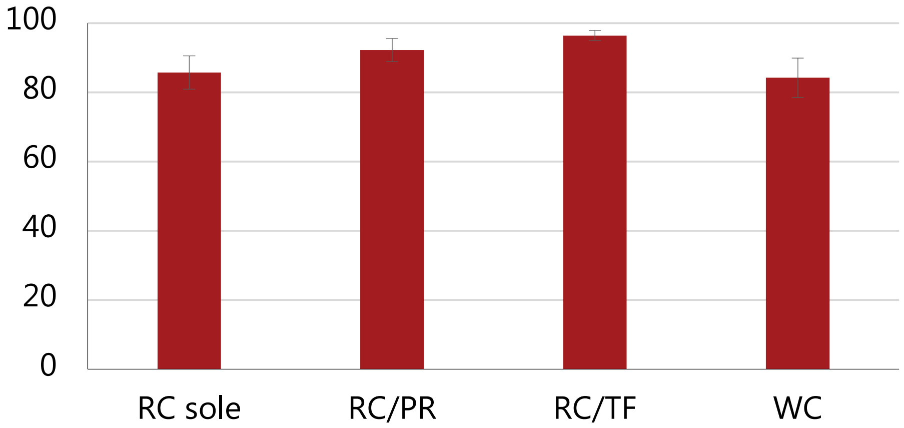
$\delta^{15}N$_legumes_Oct 2022



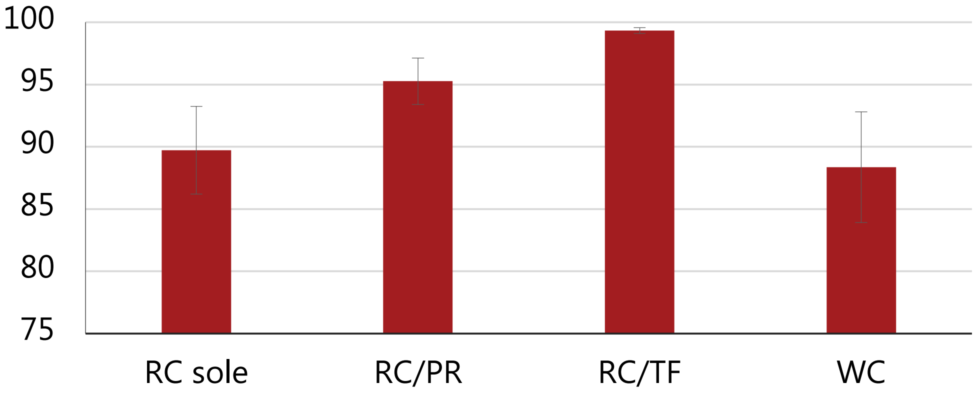
$\delta^{15}N$_legumes_Aug, 2023



%Ndfa, Oct 2022



%Ndfa_Aug 2023

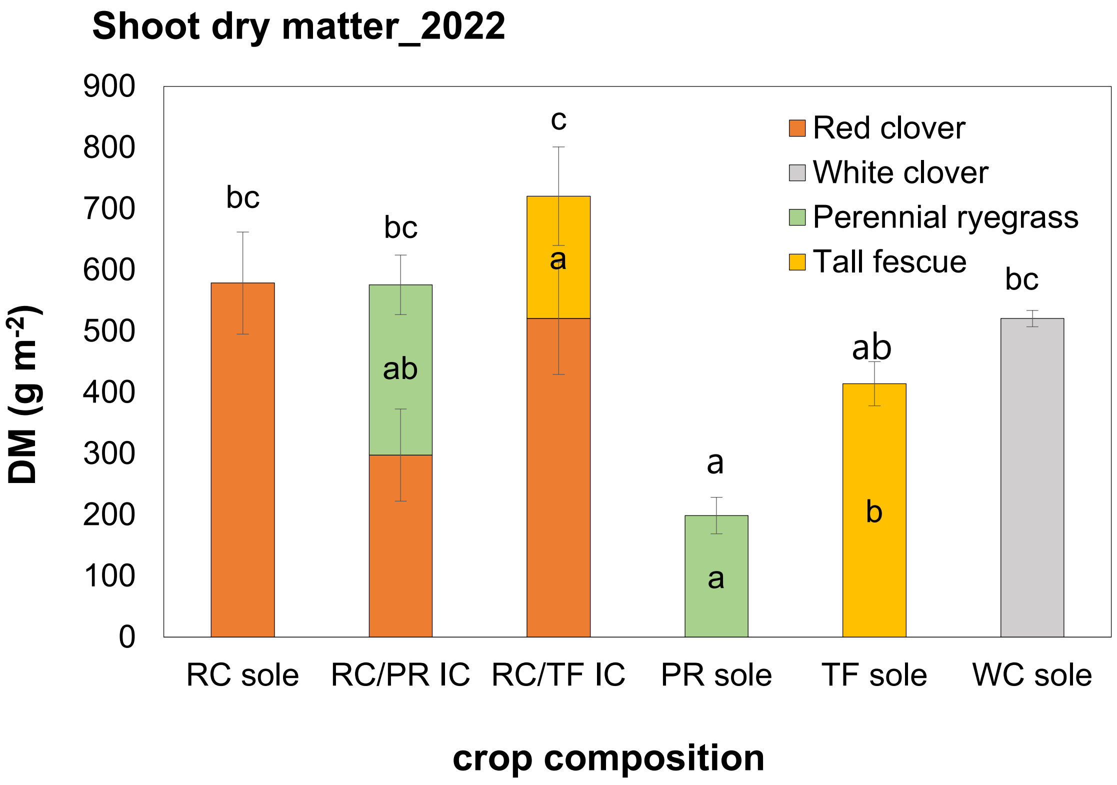


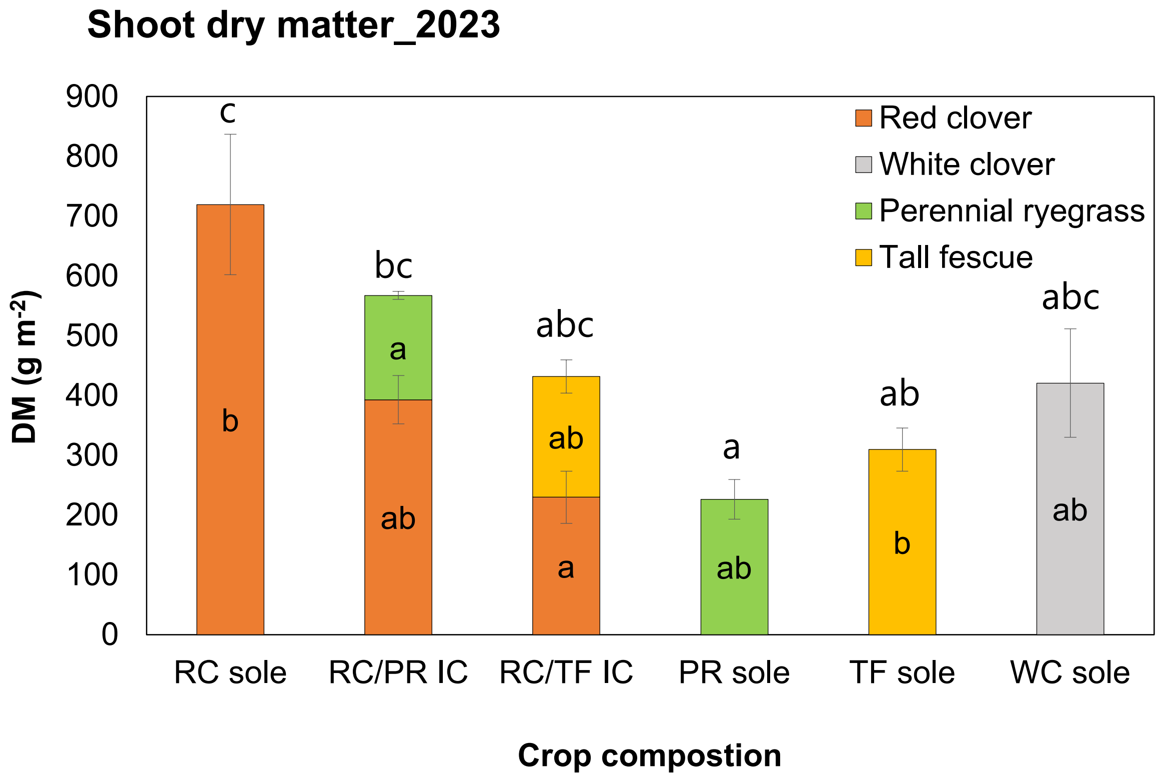
%Ndfa: Percentage of N derived from atmospheric N2-fixation estimation

RC: red clover; **WC:** white clover; **PR:** Perennial ryegrass; **TF:** Tall fescue

Results: Root tower experiment (WP3)

Plant production

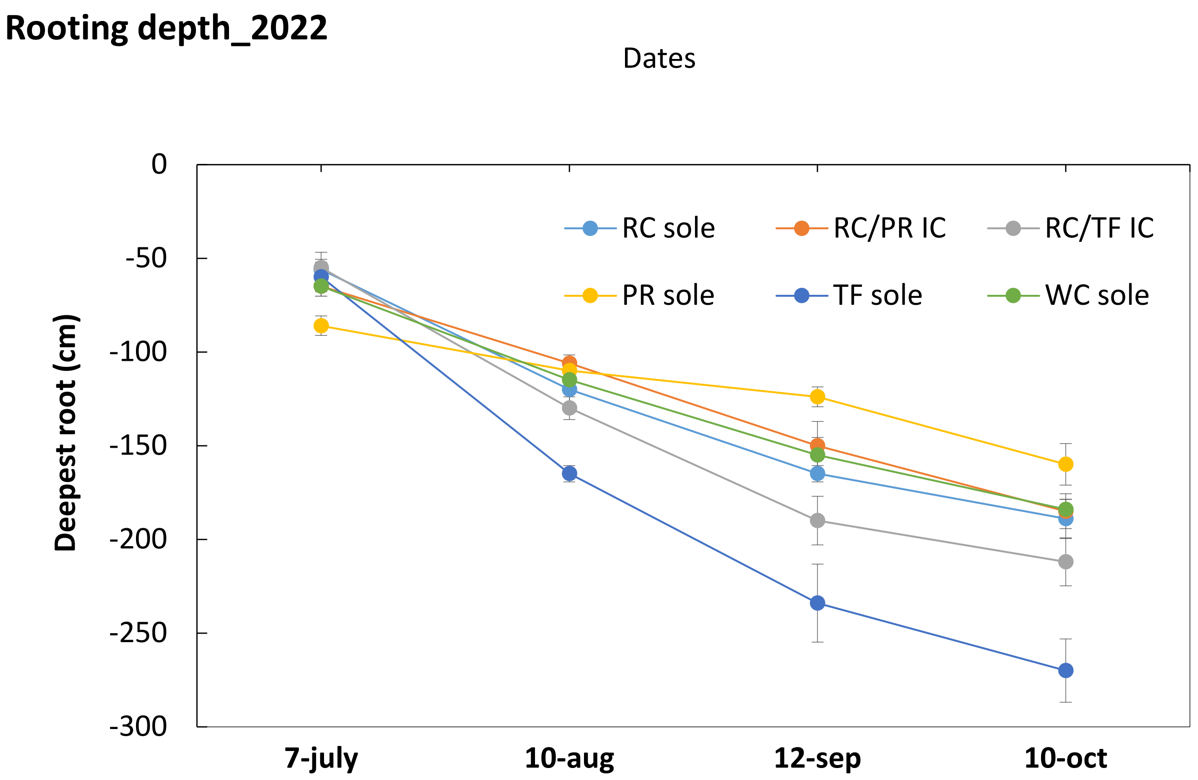




RC: Red clover; WC: White clover; PR: Perennial ryegrass and TF: Tall fescue

Results: Root tower experiment (WP3)

Root depth (deepest root observed)



Oral and Poster presentation at the International Legume Society Conference **(ILS4)**, 2023 in Granada, Spain

UNIVERSITY OF COPENHAGEN
DEPARTMENT OF PLANT AND ENVIRONMENTAL SCIENCES

Cultivar differences in root development and depth of forage legumes

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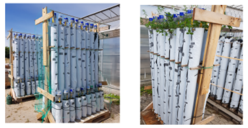
Corresponding author: njr@plen.ku.dk

Introduction

Root system development and depth are key to efficient resource use and improved crop agronomic and environmental performances. However, forage legumes are not bred for the belowground traits, and knowledge of cultivar differences in root system development and function is lacking. We investigate cultivar differences in root growth and depth over time of the three important forage legumes: red clover (*Trifolium pretense* L.), white clover (*Trifolium repens* L.), and lucerne (*Medicago sativa* L.)

Methods

Rhizotube experiment
2 m and 0.5 m tall and Ø: 10 cm

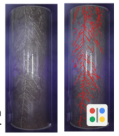


Measurements
- Early establishment
- Root growth development
- Root image analysis with RootPainter[1] software
- Root system architecture

Tubes at crop harvest at 118 days after transplanting (DAT)

Cultivars

Lucerne (LU)	Red clover (RC)	White clover (WC)
Cigale	Elara	Silvester
Musette	Amos	DLF TRF 3536
Mezzo	Callisto	Brianna



Root segmentation with RootPainter

Results

(1) Early root system architecture

Figure 1: *Roots recovered and washed in 0.5 m rhizotron tubes at 28 DAT*

(2)

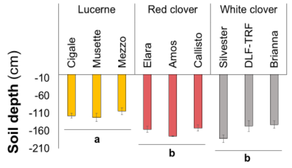


Figure 2: *Maximum root depth at 118 DAT*

(1) Faster initial root growth of Lucerne
(**Lucerne** > **Red clover** > **White clover**)

(2) Maximum root depth at DAT 118:
White clover = **Red clover** > **Lucerne**.
The deepest rooting depth of the white clover cultivar Silvester (1.82 m)

(3) Larger pRLD of Red and White clover compared to Lucerne at soil depths between 0.3 and 1.2 m (p<0.001)
RC-Elara > **WC-DLF TRF** > **RC-Amos**

(3)

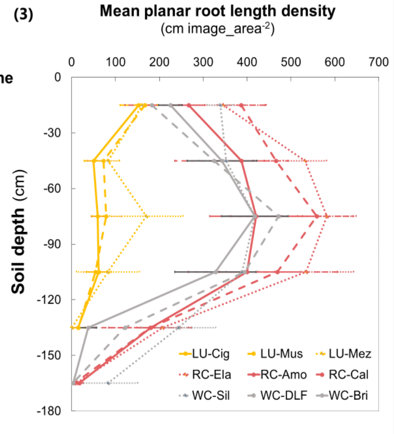


Figure 3: *Planar root length density determined from the root images taken in 2 m rhizotron tubes at 118 DAT*

Conclusions

Contrary to the general perception, our results showed **deep root growth** of **white clover**. The study provides insights into the **deep-root development** of three forage legumes, with **implications for future breeding**. The rhizotron study is currently being **validated** in the **field** and in **4 m tall rhizoboxes**, including **root function** studies based on **tracer uptake**.

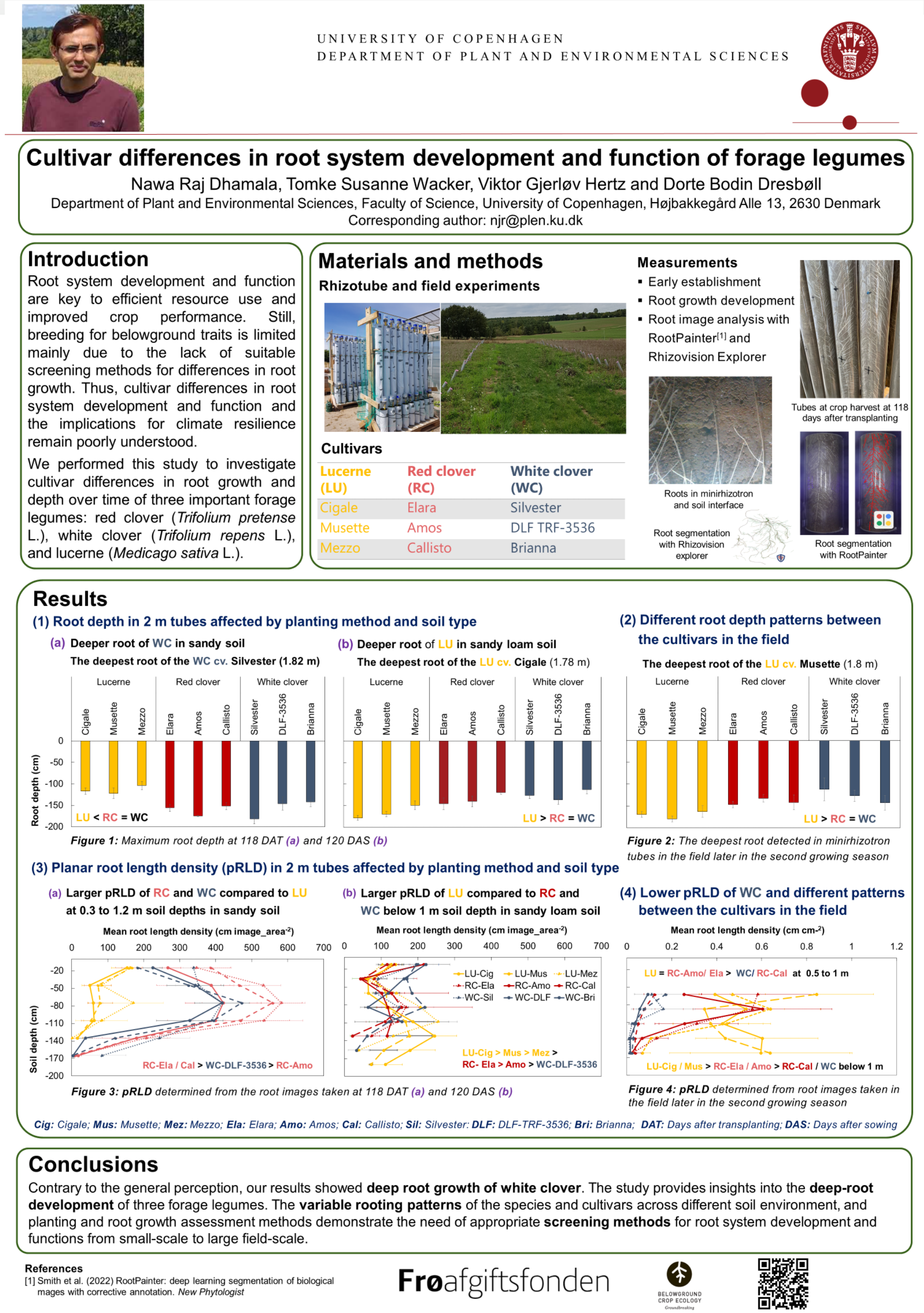
References

[1] Smith et al. (2022) RootPainter: deep learning segmentation of biological mages with corrective annotation. *New Phytologist*

Frøafgiftsfonden

BELOWGROUND CROP ECOLOGY

Poster presentation at the International Society of Root Research 12th International Symposium (12 ISRR 2024) in Leipzig, Germany


UNIVERSITY OF COPENHAGEN
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Cultivar differences in root system development and function of forage legumes

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Introduction

Root system development and function are key to efficient resource use and improved crop performance. Still, breeding for belowground traits is limited mainly due to the lack of suitable screening methods for differences in root growth. Thus, cultivar differences in root system development and function and the implications for climate resilience remain poorly understood.

We performed this study to investigate cultivar differences in root growth and depth over time of three important forage legumes: red clover (*Trifolium pretense* L.), white clover (*Trifolium repens* L.), and lucerne (*Medicago sativa* L.).

Materials and methods

Rhizotube and field experiments

Cultivars

Lucerne (LU)	Red clover (RC)	White clover (WC)
Cigale	Elara	Silvester
Musette	Amos	DLF TRF-3536
Mezzo	Callisto	Brianna

Measurements

- Early establishment
- Root growth development
- Root image analysis with RootPainter[1] and Rhizovision Explorer

Tubes at crop harvest at 118 days after transplanting

Roots in minirhizotron and soil interface

Root segmentation with Rhizovision explorer

Root segmentation with RootPainter

Results

(1) Root depth in 2 m tubes affected by planting method and soil type

(a) Deeper root of WC in sandy soil
The deepest root of the WC cv. Silvester (1.82 m)
LU < RC = WC

(b) Deeper root of LU in sandy loam soil
The deepest root of the LU cv. Cigale (1.78 m)
LU > RC = WC

Figure 1: Maximum root depth at 118 DAT (a) and 120 DAS (b)

(2) Different root depth patterns between the cultivars in the field

The deepest root of the LU cv. Musette (1.8 m)
LU > RC = WC

Figure 2: The deepest root detected in minirhizotron tubes in the field later in the second growing season

(3) Planar root length density (pRLD) in 2 m tubes affected by planting method and soil type

(a) Larger pRLD of RC and WC compared to LU at 0.3 to 1.2 m soil depths in sandy soil
RC-Ela / Cal > WC-DLF-3536 > RC-Amo

(b) Larger pRLD of LU compared to RC and WC below 1 m soil depth in sandy loam soil
LU-Cig > Mus > Mez > RC- Ela > Amo > WC-DLF-3536

Figure 3: pRLD determined from the root images taken at 118 DAT (a) and 120 DAS (b)

(4) Lower pRLD of WC and different patterns between the cultivars in the field

LU = RC-Amo/ Ela > WC/ RC-Cal at 0.5 to 1 m
LU-Cig / Mus > RC-Ela / Amo > RC-Cal / WC below 1 m

Figure 4: pRLD determined from root images taken in the field later in the second growing season

Cig: Cigale; Mus: Musette; Mez: Mezzo; Ela: Elara; Amo: Amos; Cal: Callisto; Sil: Silvester: DLF: DLF-TRF-3536; Bri: Brianna; DAT: Days after transplanting; DAS: Days after sowing

Conclusions

Contrary to the general perception, our results showed **deep root growth of white clover**. The study provides insights into the **deep-root development** of three forage legumes. The **variable rooting patterns** of the species and cultivars across different soil environment, and planting and root growth assessment methods demonstrate the need of appropriate **screening methods** for root system development and functions from small-scale to large field-scale.

References
[1] Smith et al. (2022) RootPainter: deep learning segmentation of biological mages with corrective annotation. *New Phytologist*

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