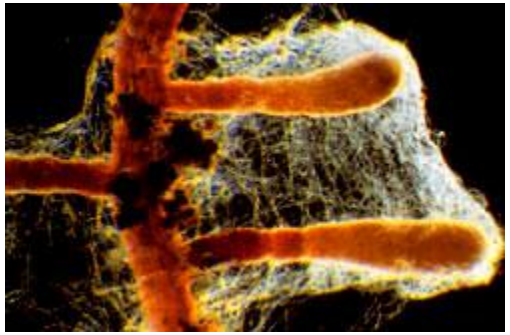
Paprastosios pušies šeimų sėjinukų augimo ypatybės azoto ir mikorizės poveikyje

Growth peculiarities of seedlings of Scots pine families under nitrogen and mycorrhiza impact



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LRCAF INSTITUTE OF FORESTRY

Project FAIR5-CT97-3454 NUTRIGEN- Exploitation of Nutrient Efficiency in Forest Tree Breeding

Subtask: Genetic variation in growth and biomass traits of half-sib families of *Pinus sylvestris* L. in mycorrhiza x nitrogen factorial experiment in phytotrone

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Introduction

- The wide distribution of *Pinus sylvestris* throughout Europe and Asia exposes this species to a variety of environmental conditions. Therefore, there is a need to understand the genetic basis of adaptive traits in relation to nutrient availability, utilization efficiency and allocation.
- Due to mycorrhizal mutualistic association between plants and fungus the plant benefits of the mycelium's higher absorptive capacity for water and mineral nutrients due to the much large surface area of mycelium
- Due to myhorrhiza fungus gains relatively constant and direct access to carbohydrates.
- 95% of plant families are predominantly mycorrhizal

Aim of the study

•To assess genetic variation, genotype x environment interactions, and phenotypic plasticity for traits of adaptive significance - juvenile growth and biomass distribution in open-pollinated families of *Pinus sylvestris* under different nitrogen availability: combinations of nitrogen regimes with presence or absence of mycorrhiza.

•To elaborate methods for evaluation stability and reaction norms of open-pollinated families in order to identify genotypes that grow well under limited resources of nutrients as well as genotypes that benefit from fertile site

Ectomycorrhiza: *Lacaria bicolor* on roots

Ectomycorrhizal seedlings of *Pinus sylvestris*

Methods and material (1)

Material

The plant material used phytotrone experiment were 9 open-pollinated families of *Pinus sylvestris* from seed orchard FP-49 located in Ekebo southern Sweden (latitude 56°00'N, altitude 85m. a s l). Clones originates from southern Swedish populations (lat. 56°48' – 57°49' N, long. 14°12'– 15°55' E, and between the altitudes 100 and 230 m a s l)

Methods and material (2)

Seedlings of *Pinus sylvestris* were grown for 13 weeks in climatic chambers

4 nitrogen regimes – 10, 25, 40, and 55 mg /l N

in combination with or without ectomycorrhizal fungus *Lacaria bicolor*.

The factorial combination of nitrogen and mycorrhiza resulted in 8 treatments:
M10N, 10N, M25N, 25N, M40N, 40N, M55N, 55N.

Methods and material (3)

Cultivation in growth chambers (1)

Randomised complete block design was used with single-tree plots with 1 seedling in each of 22 blocks per family in each treatment.

Totally 1584 seedlings were grown.

Seed were sown in single plastic tubes (diameter 26 mm, volume 79.6 cm^3). The tubes were filled with mixture of fine gravel, sand, perlite and vermiculite (8:4:3:15). The tubes were placed on trucks with 120 tubs per truck.

In climatic chambers progeny were grown for 6 weeks. Then transplanted into 1.7 litre pots filled with pumice. Containers were put into boxes and placed on trucks with 9 containers per truck.

Methods and material (4)

Cultivation in growth chambers (2)

During transplanting part of plants for mycorrhizal association treatment were inoculated by dipping roots in a suspension of fungal mycelium before potting (Kahr&Arveby 1986).

Inoculum was prepared from six-week old fungal mycelia cultivated in Pachlewski liquid culture(Pachlewski and Pachlewski 1974). The species used for inoculation was the ectomycorrhizal symbiont ***Lacaria bicolor*** (Maire) Orton, strain 238. This species was chosen for its ability to produce extraradical mycelium with many tree species at higher nitrogen concentrations (Wallander and Nylund 1992).

Methods and material (5)

Cultivation in growth chambers (3)

During the subsequent two weeks the pots with plants were watered every 3 days with balanced nutrient solution: 100 N, 84.3 K, 19.6 P, 5.9 Ca, 7.8 Mg, 7,8 S, and microelements according to Ingestad and Lund (1986). The plants were watered to saturation supplying solution from above of the containers by filling the large boxes with solution up to 10 cm level for 30 minutes, then allowing all residual water be drained away.

Methods and material (6)

Cultivation in growth chambers (4)

During the next 5 week period plant were grown without any further fertilization to allow plants to express their genetic differences in ability to exploit nutrients available in the pots.

In order to avoid drought the large volume pots were used, surface of substrate was covered with plastic and high relative air humidity was kept in the chambers.

The seedlings were grown in light from daylight lamps providing an irradiance of about 400 µ mol•m-2•s-1 in the 400-700 nm spectrum. The photoperiodic regime was intended to mimic the conditions during vegetation period

Methods and material (7)

Assesments

After one growth period (13 weeks) **seedling height** was measured, the **length of the elongated hypocotyl** was measured as an estimate of the initial growing capital to use as covariate in the analysis.

Seedlings were harvested. **Dry weight** was measured after drying to constant weight in oven for 40 hours at 70^{o}C. The plant **dry weights of stem (SDW)** and **root (RDW)** were recorded separately.

Total dry weight (TDW), root/stem dry weight ratio (RSDW) were derived from the assessed individual weights.

The damages were scored 1 to 3.

The occurrence of buds on each seedling was recorded.

Methods and material (8)

Statistical analysis

In order to diminish the influence of significant differences in seed size elongated hypocotyl length was used as covariate.

Variance analysis was done using MIXED procedure in the SAS Software (Release 6.12). Mixed model equations (MME) and the restricted maximum likelihood (REML) method were used for computing variance components.

Methods and material (9)

Statistical analysis

The following linear models used for joint analyses of eight treatments together and for separate analyses of individual treatments:

1) Joint: $$y_{ijkl} = \mu + ah_{ijkl} + b_i(mn) + m_j + n_k + f_l + fm_{jl} + fn_{lk} + fmn_{ljk} + \varepsilon_{ijkl}$$

2) Separate: $$y_{il} = \mu + ah_{il} + b_i + f_l + \varepsilon_{il}$$

where y_{ijkl} and y_{il} - values of single observation, μ - grand mean, a - regression coefficient, h_{ijkl} - fixed effect of elongated hypocotyl length for individual *ijkl*, m_j - fixed effect of mycorrhiza association *j*, n_k - fixed effect of nutrient regime *k*, $b_i(mn)_{jk}$ - fixed effect of block *i* within treatment *jk*, b_k - fixed effect of block *i*, f_l - random effect of family *l*, fm_{lj} - random effect of interaction between family *l* and mycorrhiza association *j*, fn_{lk} - random effect of interaction between family *l* and nutrient regime *k*, fmn_{ljk} - random effect of interaction between family *l*, nutrient regime *k* and mycorrhiza association *j*, e_{ijkl} and e_{il} - random error

Methods and material (10)

Genetic parameter estimates

The families were considered as half-sibs and genetic parameters were interpreted as:

Additive genetic variance: $\sigma_A^2 = 4\sigma_f^2$

Environmental variance: $\sigma_E^2 = \sigma_e^2 - 3\sigma_f^2$

Additive genetic coefficients of variation: $CV_A = \frac{\sqrt{4 \cdot \sigma_f^2}}{\overline{X}} \cdot 100$

Individual tree heritabilities: $h_i^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_E^2}$

where σ_A^2 - additive genetic variance, σ_f^2 - family variance component of population, σ_e^2 - error variance component, $\overline{X}$ - the phenotypic mean of the trait.

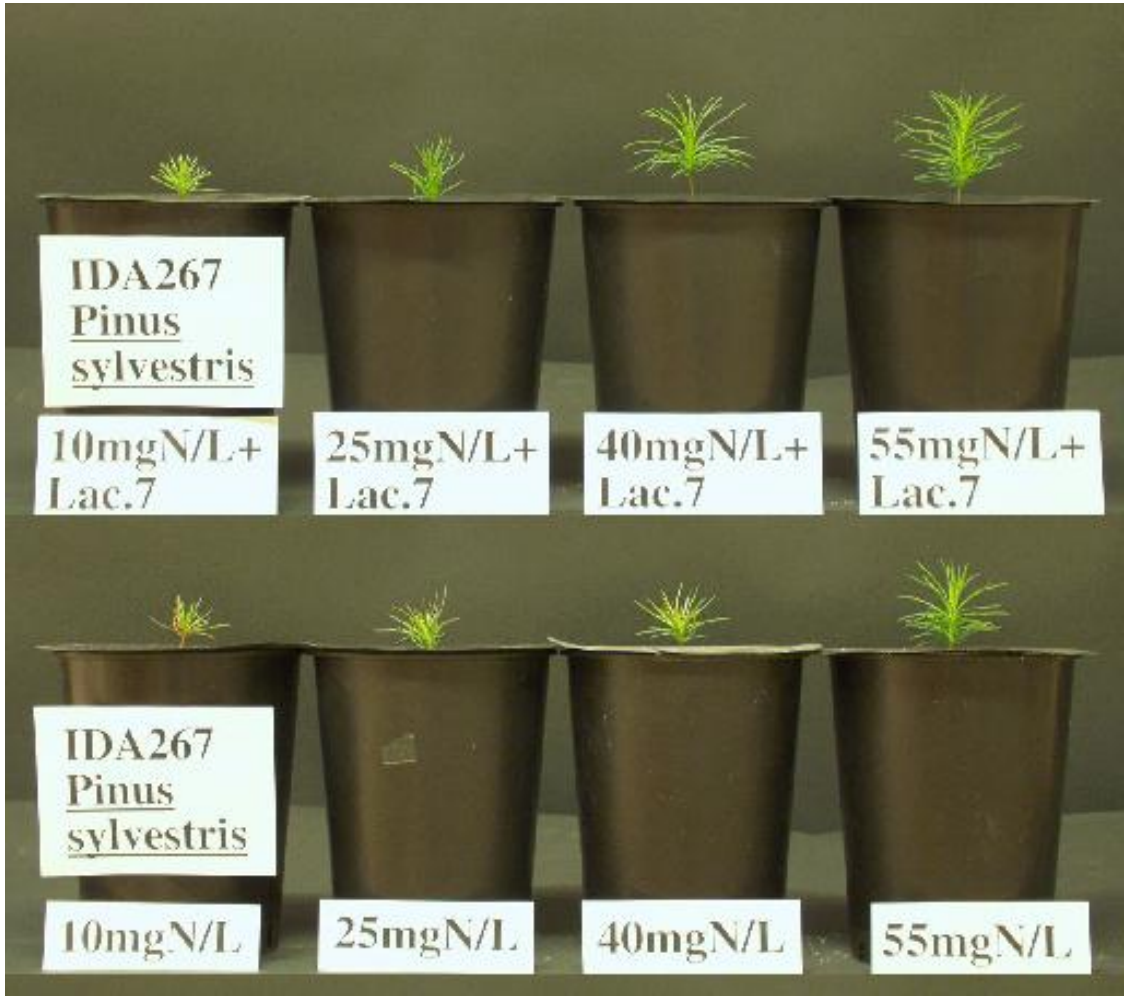
Heritabilities were calculated for each treatment separately. Standard errors of individual heritabilities were calculated as described for unbalanced designs in Becker (1984)

Methods and material (11)

Stability analysis of populations and families

To estimate the contribution of each family to the family x treatment interaction variances, the ecovalence value (Wricke 1962) of families by populations were calculated on individual observations level, using solutions to the mixed linear model (BLUP) for individual families within each treatment (Solutionr, SAS procedure MIXED). The ecovalence value as a measure of interaction variance for each family was expressed in percent of the total interaction variance. The stability variances was computed and significance of ecovalences was tested using the method developed by SHUKLA (1972).

Results



mycorrhiza

no mycorrhiza

Figure 1. Growth of seedlings at different combinations of nitrogen availability and mycorrhiza

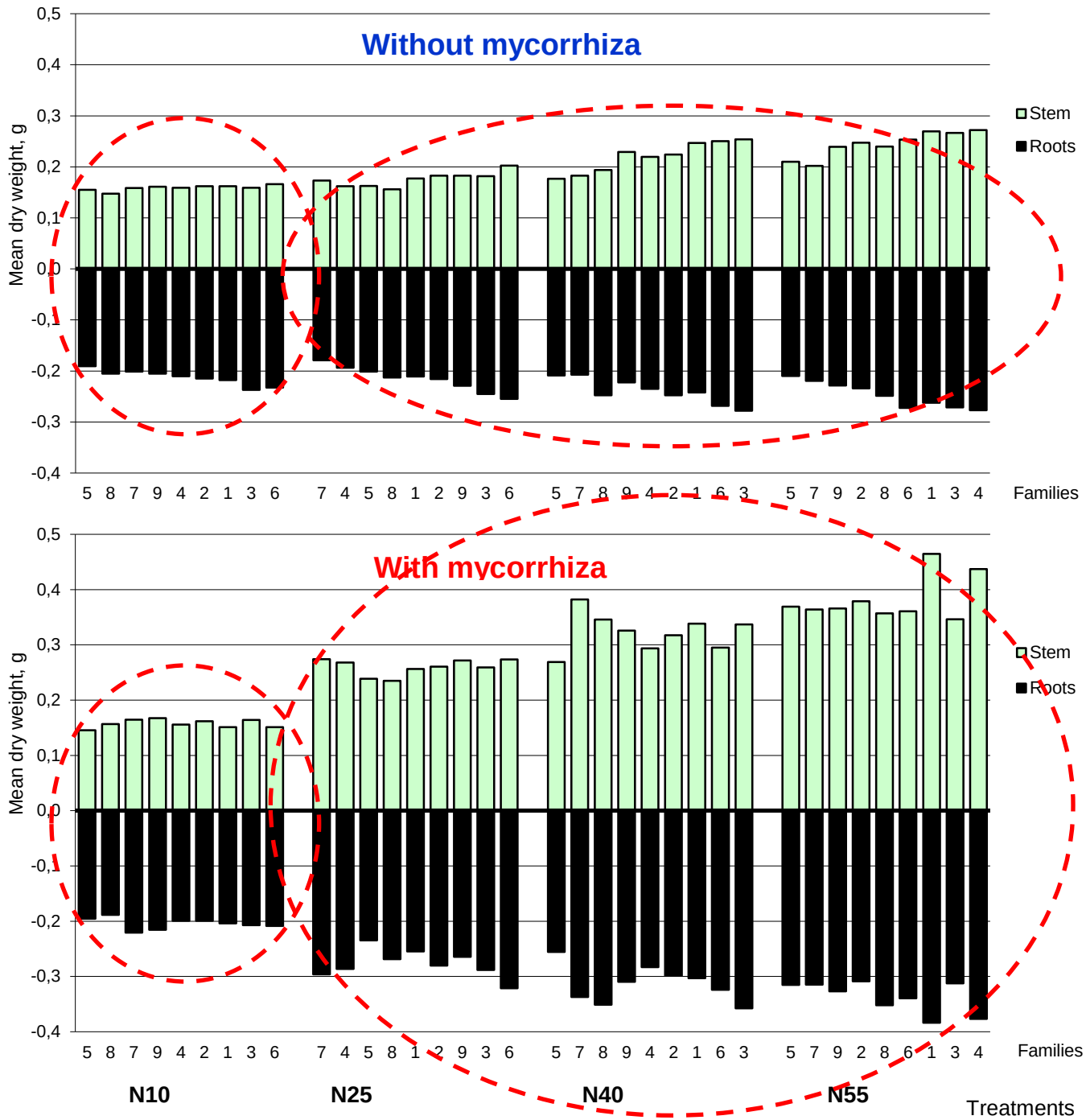


Figure 2. Distribution of biomass in seedlings of *Pinus sylvestris* families over mycorrhiza and nitrogen treatments

- Without mycorrhiza seedlings do not much benefit from nitrogen
- At low nitrogen seedlings do not benefit from mycorrhiza
- Under mycorrhiza seedlings significantly benefit from available increased nitrogen

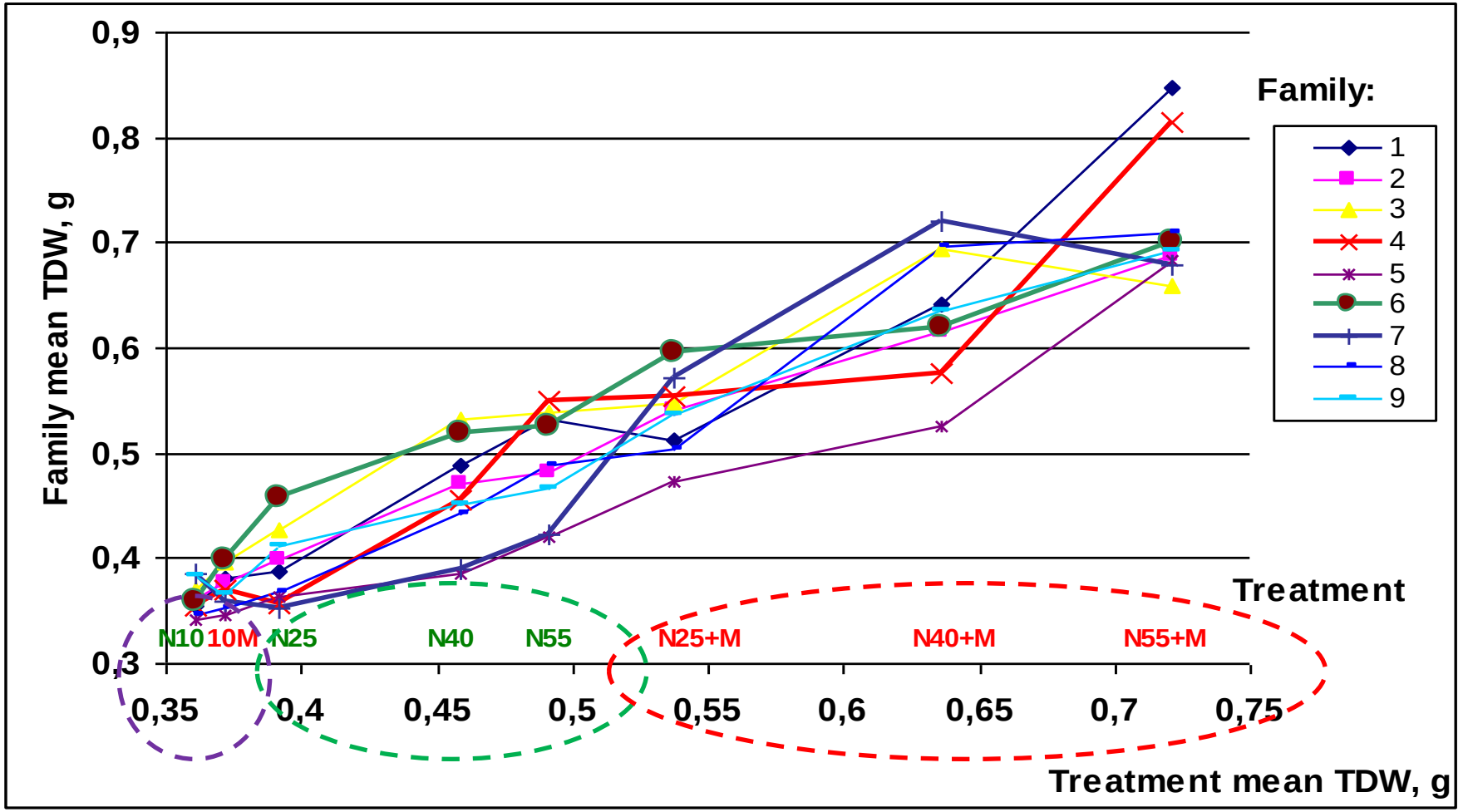


Figure 3. Variation in total biomass (TDW) of *Pinus sylvestris* open-pollinated families along soil fertility gradient (derived from different nitrogen treatments in combination with absence or presence of mycorrhiza)

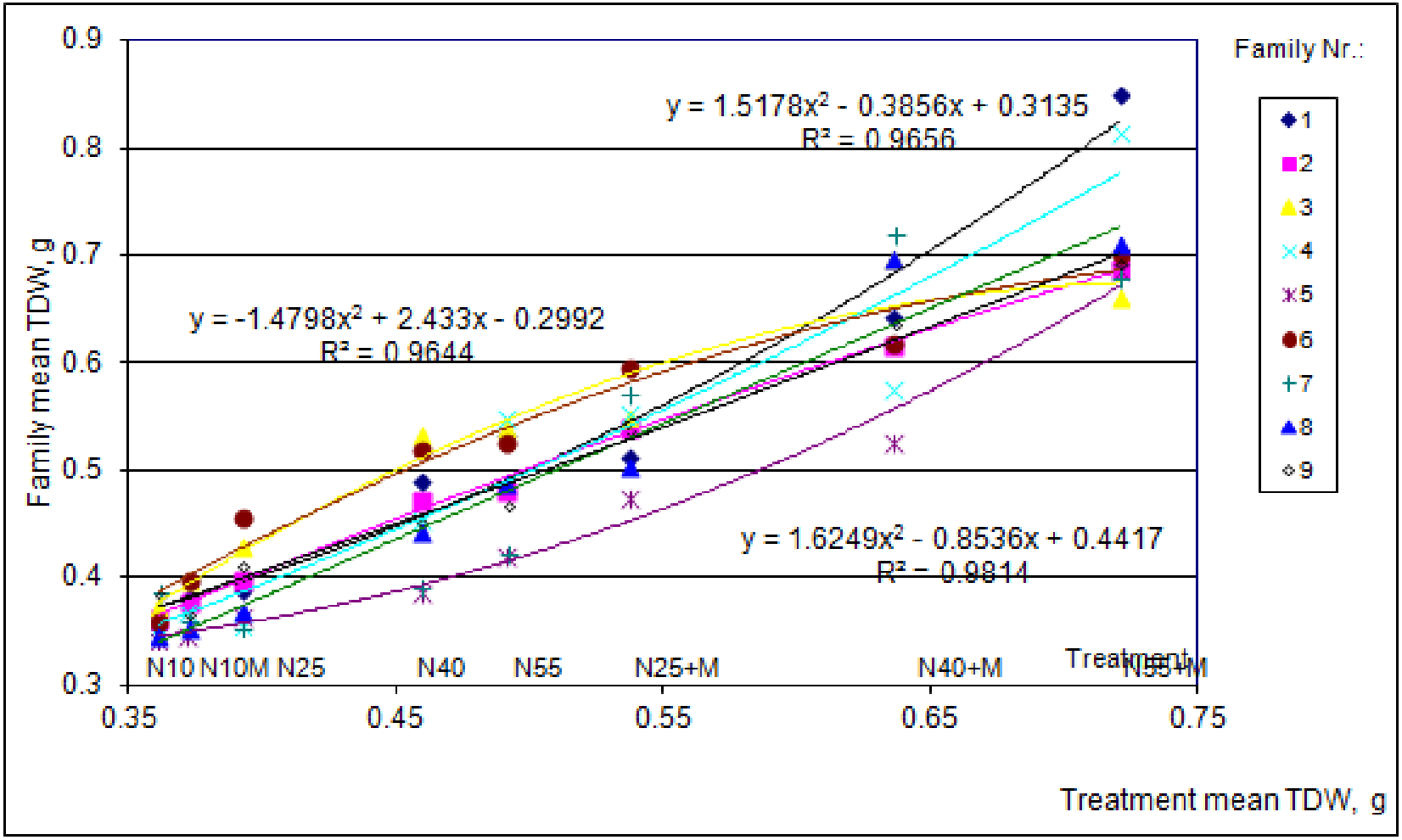


Figure 4. Reaction norms in total biomass (TDW) of *Pinus sylvestris* open-pollinated families along soil fertility gradient (derived from different nitrogen treatments in combination with absence or presence of mycorrhiza)

Table 1. Results from joint mixed linear model analysis of variance: variance components for random effects (family, family x block, family x mycorrhiza, and family x nitrogen interaction) as percent of the total random variation and significance of fixed effects: m, n, b, mn are the significance of mycorrhiza, nitrogen and mycorrhiza x nitrogen interaction respectively.

Trait	Variance components of random effects, %				Significance of fixed effects			
	σ^2_f	σ^2_{fb}	σ^2_{fm}	σ^2_{fn}	*m*	*n*	*b*	*mn*
Hypocotyls length	22.8*	0	0	0.2			***	
Seedling height	3.2*	1.2	2.8	3.3*	***	***		***
Stem dry weight	0	0	2.6	3.8*	***	***	***	***
Root dry weight	1.7	0	2.4	0	***	***	***	***
Total dry weight	0	0	3.2	4.2*	***	***	***	***
Root/stem ratio	2.5	0	0.2	0	***	***	***	*
Setting of bud	0.4	0	3.7	0.3		***	***	

Level of significance is denoted by: *- 0.05>P>0.01, ** - 0.01> P>0.001, *** - P<0.001.

Table 2. Means, individual heritabilities and additive genetic coefficients of variation for individual treatments for growth and biomass traits of *Pinus sylvestris* progenies

Trait	Nitrogen treat-ment	Without mycorrhiza				With mycorrhiza			
		Mean	h_i^2	±se	CV_A (%)	Mean	h_i^2	±se	CV_A (%)
Seedling height, mm (H)	10N	41.55	0.29	±0.21	2.9	43.03	0.13	±0.14	2.5
	25N	42.08	0.29	±0.21	5.0	52.42	0.37	±0.24	6.5
	40N	44.80	0.25	±0.19	6.2	54.98	0.58	±0.31	8.3
	55N	46.37	0.30	±0.21	7.4	59.53	0.45	±0.27	10.7
Stem dry weight, g (SDW)	10N	0.159	0	±0	0	0.157	0.03	±0.10	3.7
	25N	0.176	0.29	±0.21	11.0	0.260	0.04	±0.10	4.2
	40N	0.220	0.46	±0.27	21.9	0.323	0.98	±0.43	21.9
	55N	0.244	0.06	±0.11	8.4	0.383	0.43	±0.26	17.2
Root dry weight, g (RDW)	10N	0.213	0.35	±0.24	10.9	0.204	0	±0	0
	25N	0.216	0.76	±0.36	19.6	0.277	0.35	±0.23	14.1
	40N	0.240	0.24	±0.19	15.0	0.313	0.86	±0.40	20.4
	55N	0.247	0.19	±0.17	13.2	0.337	0.27	±0.20	13.2
Total dry weight, g (TDW)	10N	0.372	0.27	±0.20	7.5	0.362	0.01	±0.08	1.8
	25N	0.392	0.63	±0.33	15.1	0.537	0.19	±0.12	9.0
	40N	0.459	0.36	±0.23	17.4	0.636	1.04	±0.43	20.2
	55N	0.491	0.13	±0.14	11.3	0.721	0.39	±0.25	14.9
Root/stem ratio (RSDW)	10N	1.41	0.27	±0.20	11.8	1.35	0	±0	0
	25N	1.27	0.38	±0.24	14.1	1.09	0.19	±0.17	10.3
	40N	1.15	0.22	±0.18	12.0	0.99	0.54	±0.30	13.5
	55N	1.05	0.04	±0.10	4.9	0.91	0.22	±0.18	10.1

Table 3. Characteristic of stability of *Pinus sylvestris* open-pollinated families in nitrogen treatments in combination with absence or presence of mycorrhiza for total biomass (TDW). Characteristics indicating lowest stability are highlighted in bold red.

Family Nr.	Wricke ecovalence ,%		Shukla stability variance			Finlay-Wilkinson/ Eberhar.-Russell stability parameters			Quadratic parameters of polynomial function			
	standard	derived from breed. values	Variance	F	P	Interception	Regression coefficient	Residual variance	Interception	Regression coeff.	Second regression coeff.	Residual variance
1	11.9	12.4	0.0009	1.8	0.093	0.050	0.960	0.0022	-0.04	1.242	-0.195	0.0027
2	1.4	1.4	0.0000	0.0	0.000	-0.017	1.024	0.0002	0.04	0.860	0.113	0.0003
3	11.2	14.0	0.0010	1.9	0.071	0.018	1.031	0.0020	0.12	0.698	0.230	0.0024
4	15.2	18.2	0.0013	2.7	0.014	-0.018	1.046	0.0029	-0.38	2.186	-0.788	0.0019
5	5.4	3.7	0.0002	0.4	0.897	-0.031	0.940	0.0008	0.02	0.798	0.099	0.0010
6	12.1	15.5	0.0011	2.3	0.032	-0.020	1.105	0.0016	-0.12	1.417	-0.216	0.0019
7	21.0	25.6	0.0019	4.0	0.001	-0.033	1.011	0.0042	0.20	0.276	0.508	0.0045
8	6.4	6.7	0.0004	0.9	0.520	0.049	0.895	0.0005	0.10	0.724	0.118	0.0006
9	2.2	2.6	0.0001	0.2	0.977	0.007	0.978	0.0004	0.05	0.854	0.085	0.0005

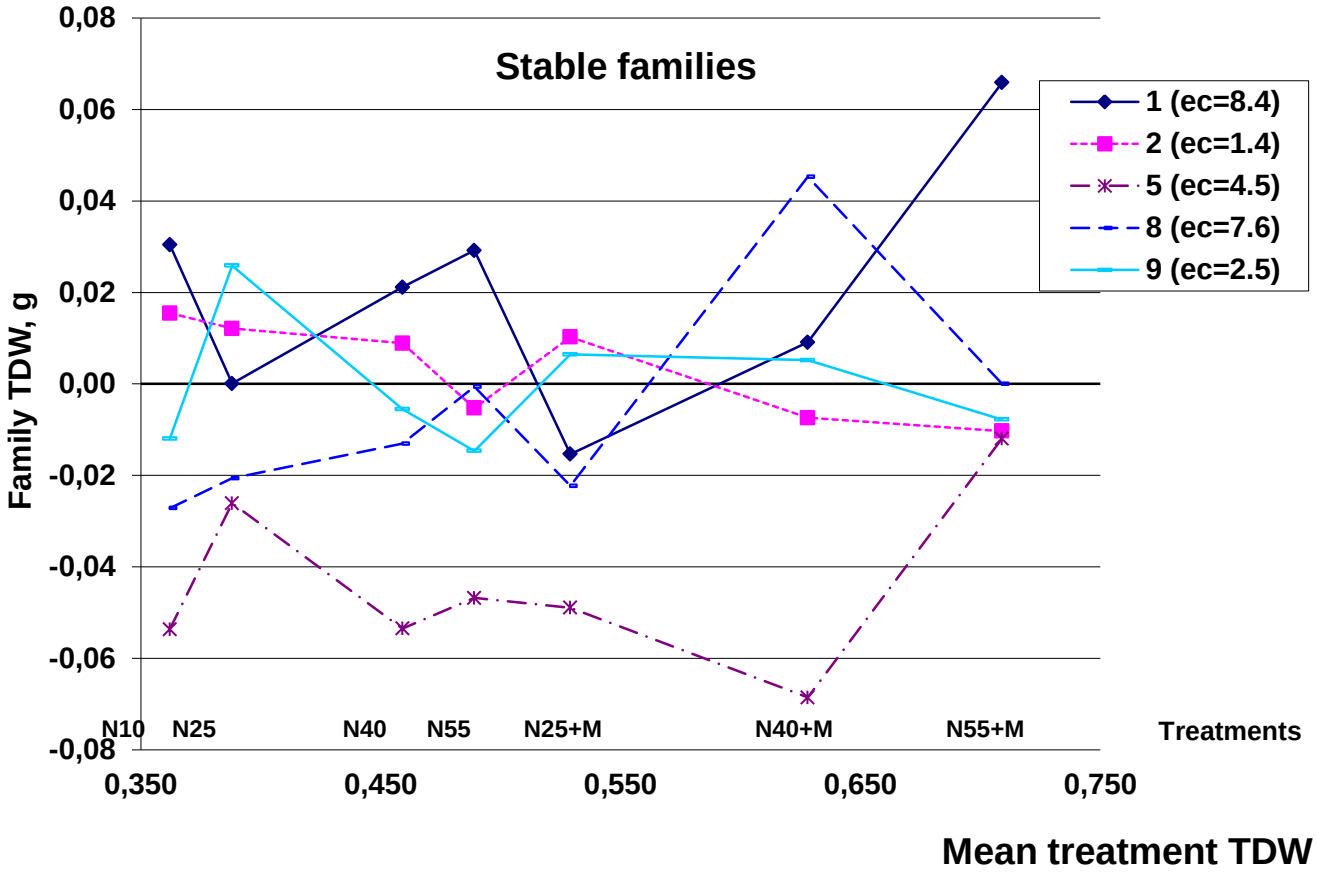


Figure 5. Relative performance of **most stable** *Pinus sylvestris* open-pollinated Families in total biomass (TDW) along soil fertility gradient (derived from different nitrogen treatments in combination with absence or presence of mycorrhiza

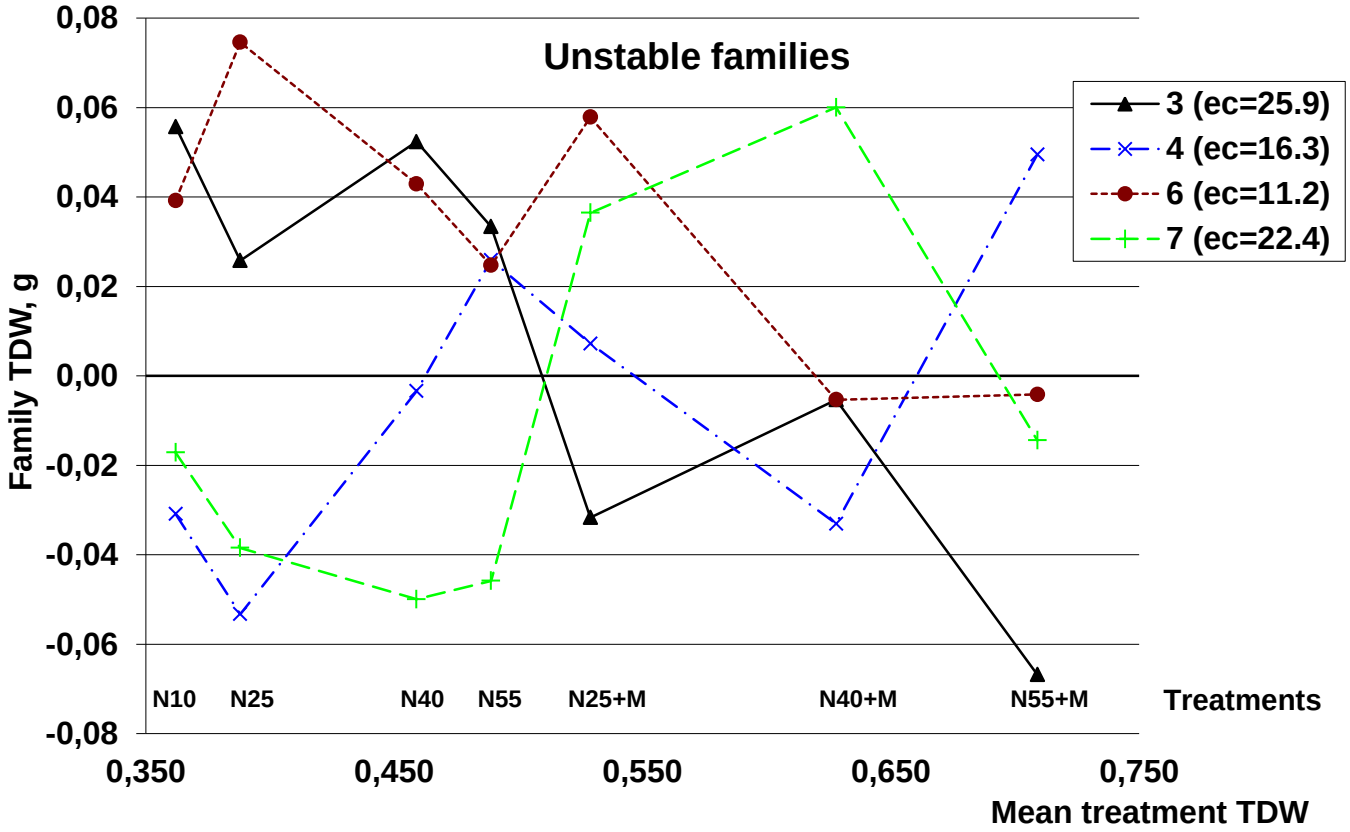


Figure 6. Relative performance of **unstable** *Pinus sylvestris* open-pollinated Families in total biomass (TDW) along soil fertility gradient (derived from different nitrogen treatments in combination with absence or presence of mycorrhiza

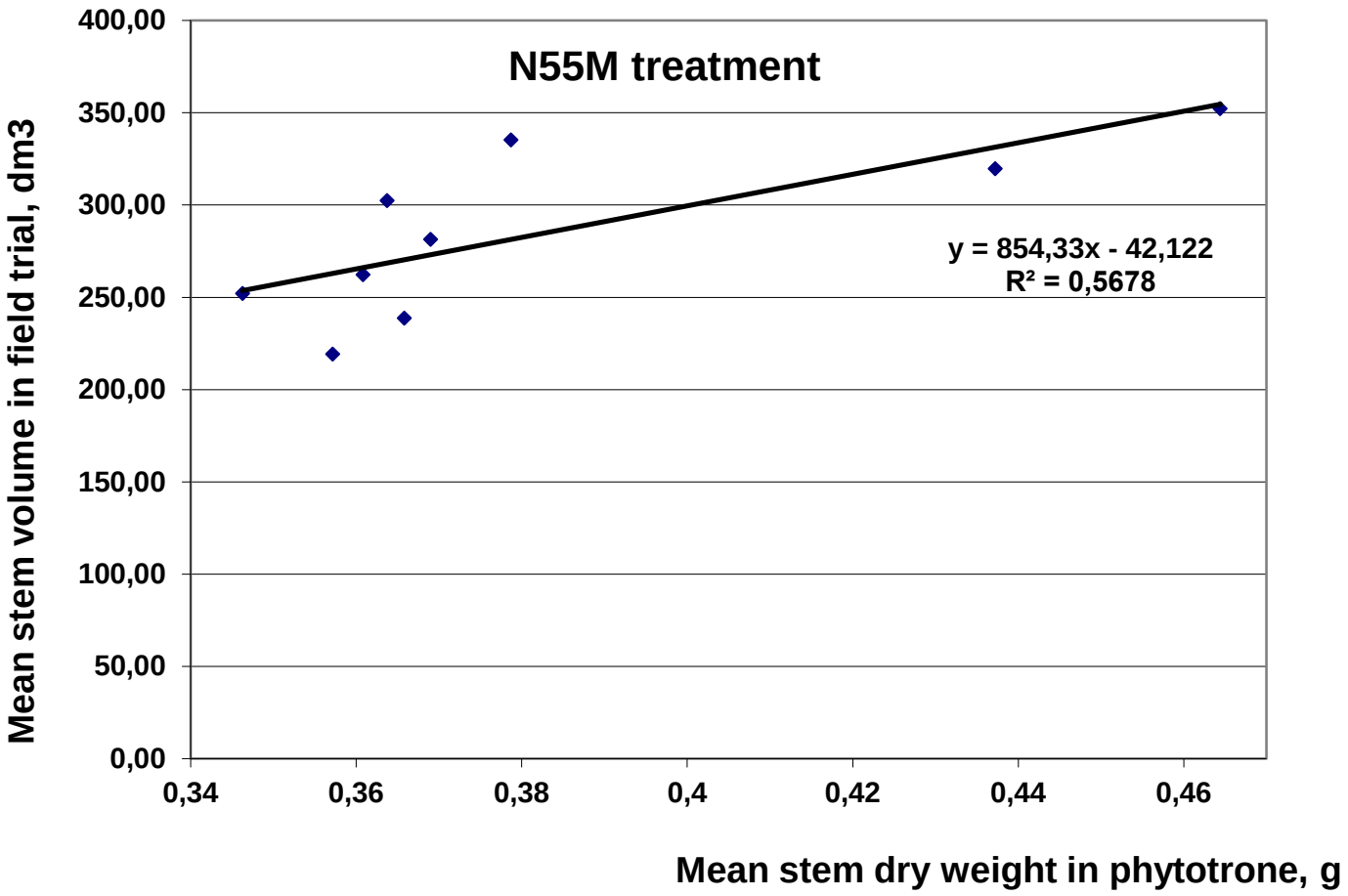


Figure 7. Regression of mean stem volume of *Pinus sylvestris* open-pollinated families in field progeny trial (established in 1964) on mean stem dry weight of the same families tested in phytotron at highest nitrogen doses (N55) with myccorhyza

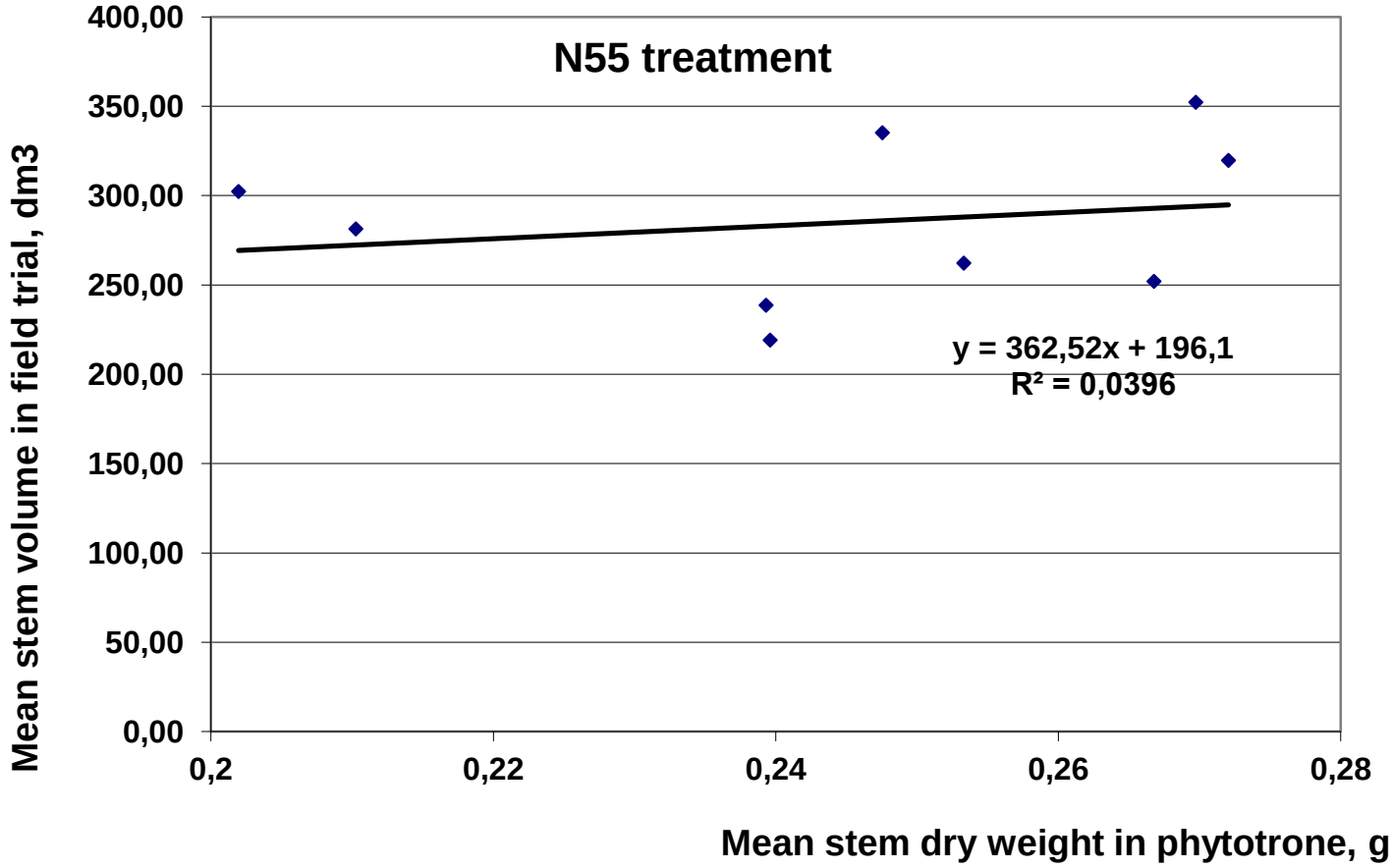


Figure 8. Regression of mean stem volume of *Pinus sylvestris* open-pollinated families in field progeny trial (established in 1964) on mean stem dry weight of the same phamilies tested in phytotron at highest nitrogen doses (N55) without myccorhyza

Conclusions

1. Effects of mycorrhiza and nitrogen were significant for most growth and biomass traits of Scots pine seedlings
2. Interaction between mycorrhiza and nitrogen were significant for most growth and biomass traits indicating that effect of nitrogen is altered by mycorrhiza and vice versa.
3. Without mycorrhiza Scots pine seedlings do not much benefit from nitrogen which shows that root system of seedlings is not well developed to absorb available larger amounts of nitrogen
4. At low nitrogen seedlings do not benefit from mycorrhiza, thus limiting factor is shortage of nitrogen but not an absorbtion abilities of mycorrhized root system
5. Under mycorrhiza seedlings significantly benefit from available increased nitrogen
6. Family effect was significant for hypocotyls length and seedling height.
7. Family by mycorrhiza interaction effect was nonsignificant meaning that families do not differ in their reaction to presence or absence of mycorrhiza.
8. Family by nitrogen interaction was significant for most growth and biomass traits except root biomass indicating, that families differ in their reaction to increased nitrogen availability