

Activity of Exoenzymes in Treated Wastewater Irrigated Soils



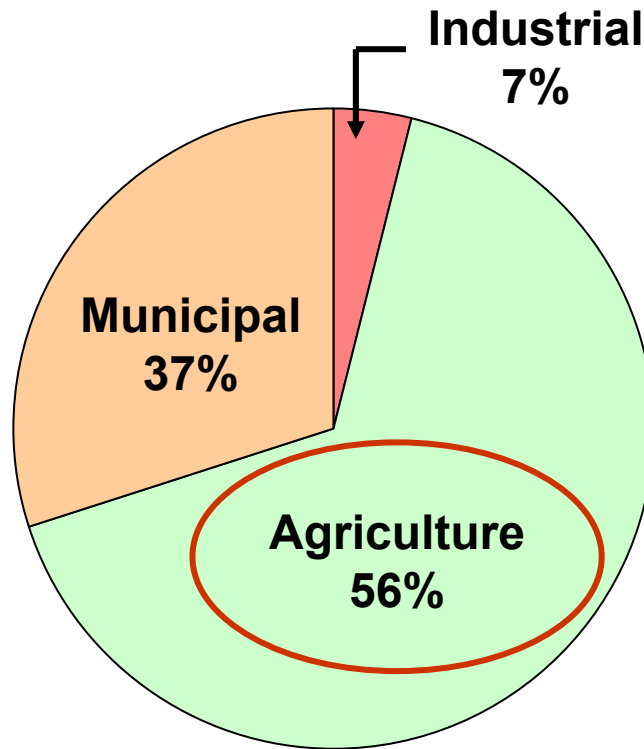
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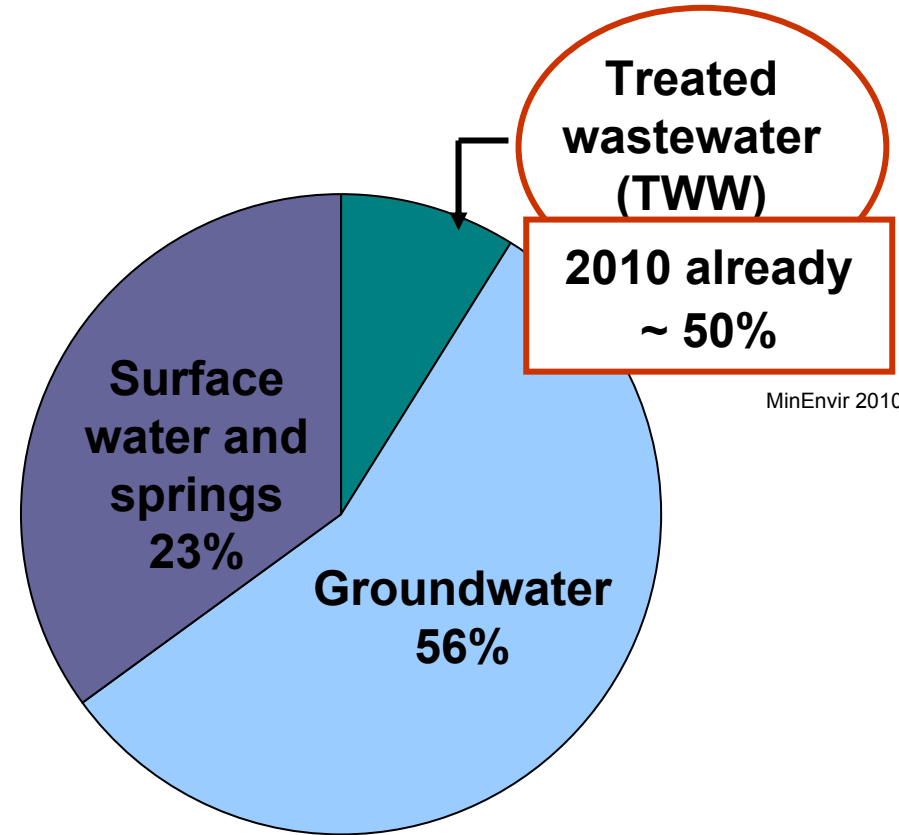
Jüschke & Marschner



Water use in Israel



Israeli Ministry of Environmental Protection (2002)

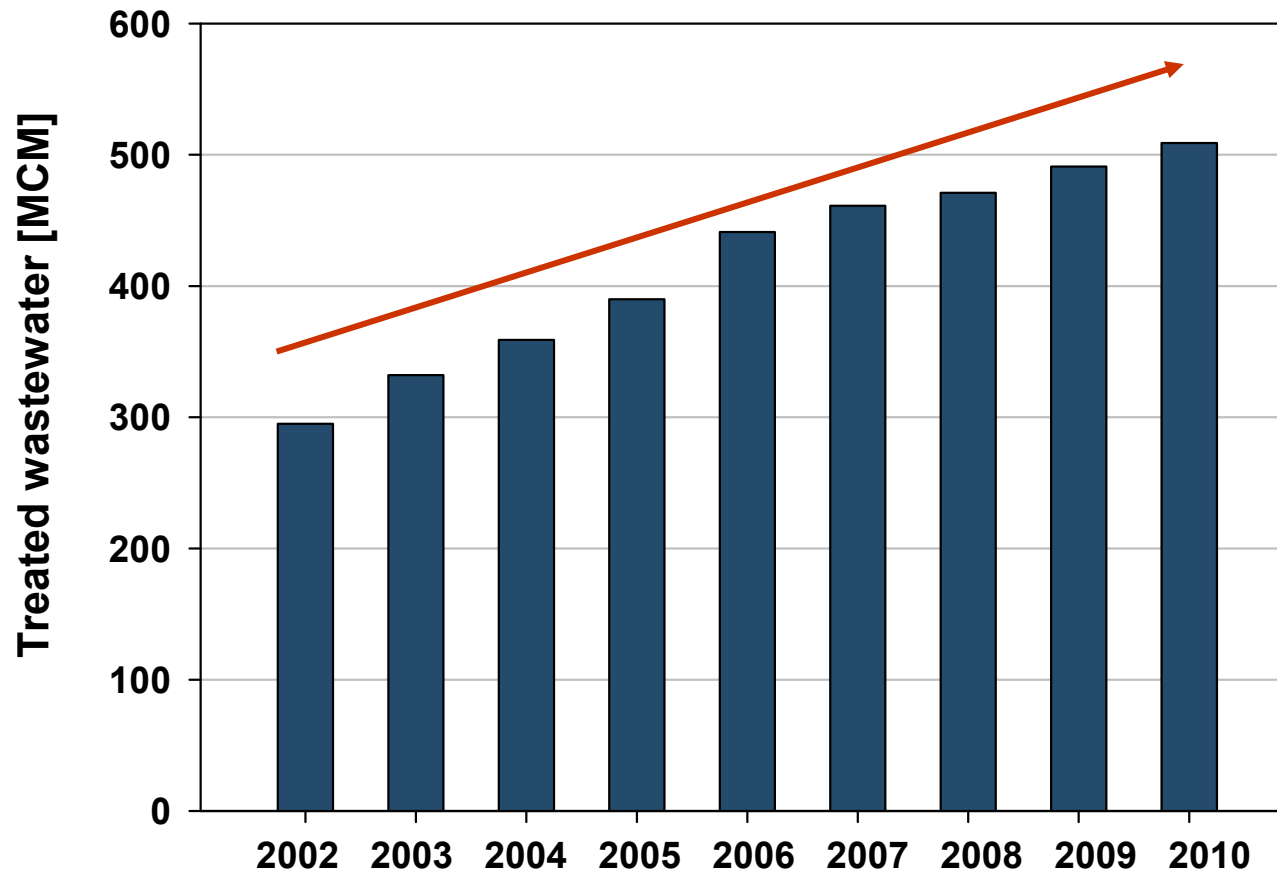


Water Commission (2000)

MinEnvir 2010

Introduction

Established and projected treated wastewater (effluent) use in Israel



MCM = million cubic meter

modified after Tal (2006)

Introduction

Water quality

Parameter	Unit	TWW	FW
EC	dS/m	2.3	1.0
Cl	mg/L	364	201
Na	meq/L	21.4	4.3
Ca + Mg	meq/L	6.1	4.1
pH		8.3	7.4
DOC	mg/L	23.5	1.1
TOC	mg/L	47.6	< 10
BOD	mg/L	59.9 [*]	< 1
COD	mg/L	234.0 ^{**}	< 20

Upgraded Effluent Quality Standards (Jan 2010)

* BOD 10 mg/L

** COD 100 mg/L

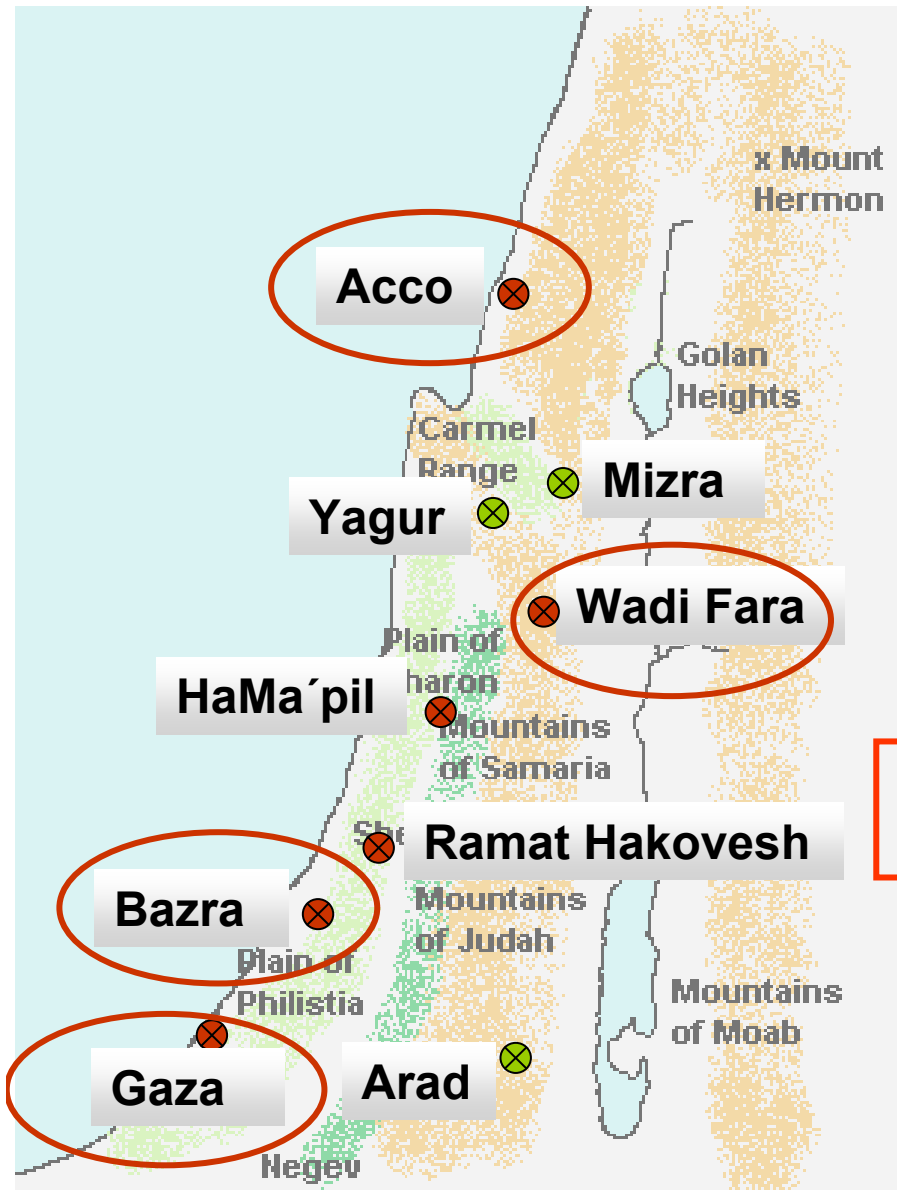
(Israel Ministry of Environment 2010)

TWW – Treated WasteWater

FW - FreshWater

Treated wastewater is a source of organic carbon.

Materials and Methods

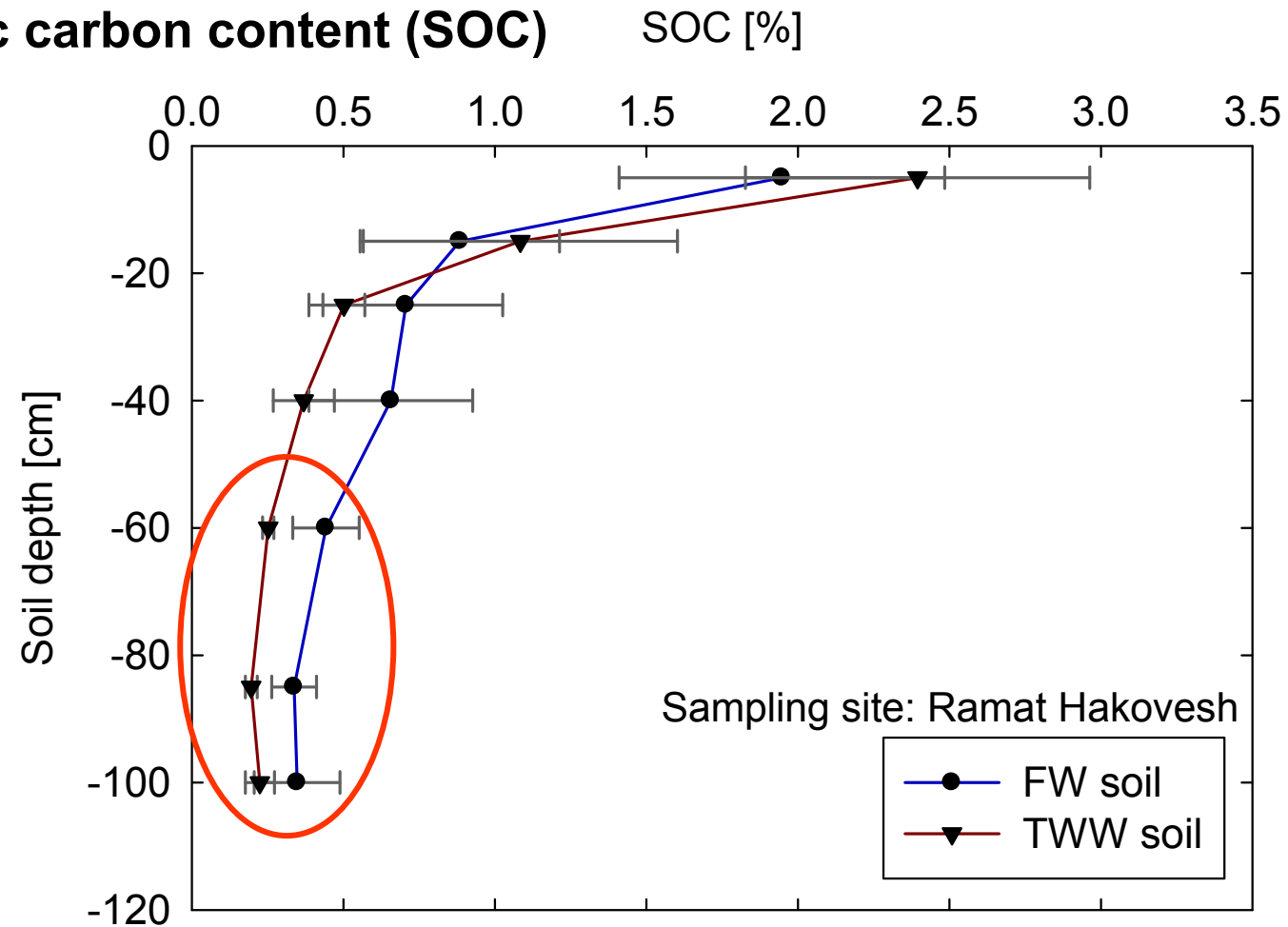


- ⊗ sampling sites
- ⊗ Soil Organic Carbon - profiles
- Enzyme activity studies

Sampling site	Land use	FAO classification	Sand (%)	Clay (%)	SOC (%)
Ramat Hakovesh	orchard	Chromic Luvisol	89	6	0.9
HaMa'pil	orchard	Chromic Luvisol	82	11	1.8
Bazra	grapefruit orchard	Chromic Luvisol	82	12	0.5
Acco	avocado orchard	Vertisol	22	52	1.0
Wadi Fara	field	Vertisol	11	66	2.3
Gaza	field	Chromic Luvisol	86	7	0.1

Results

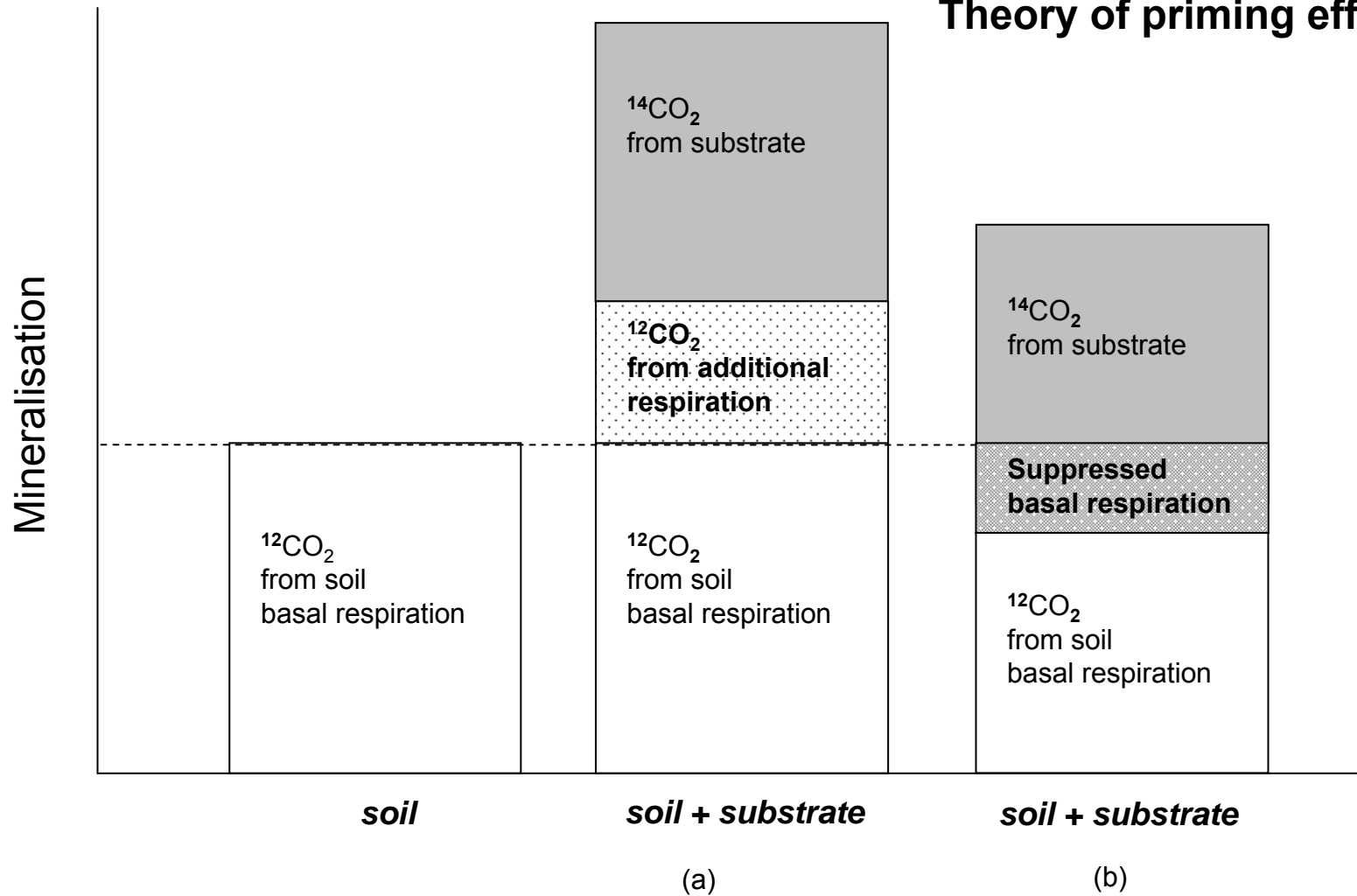
Soil organic carbon content (SOC)



- first ~50 cm similar SOC-content
- less SOC in the subsoil (deeper ~50 cm)

Materials and Methods

Theory of priming effects



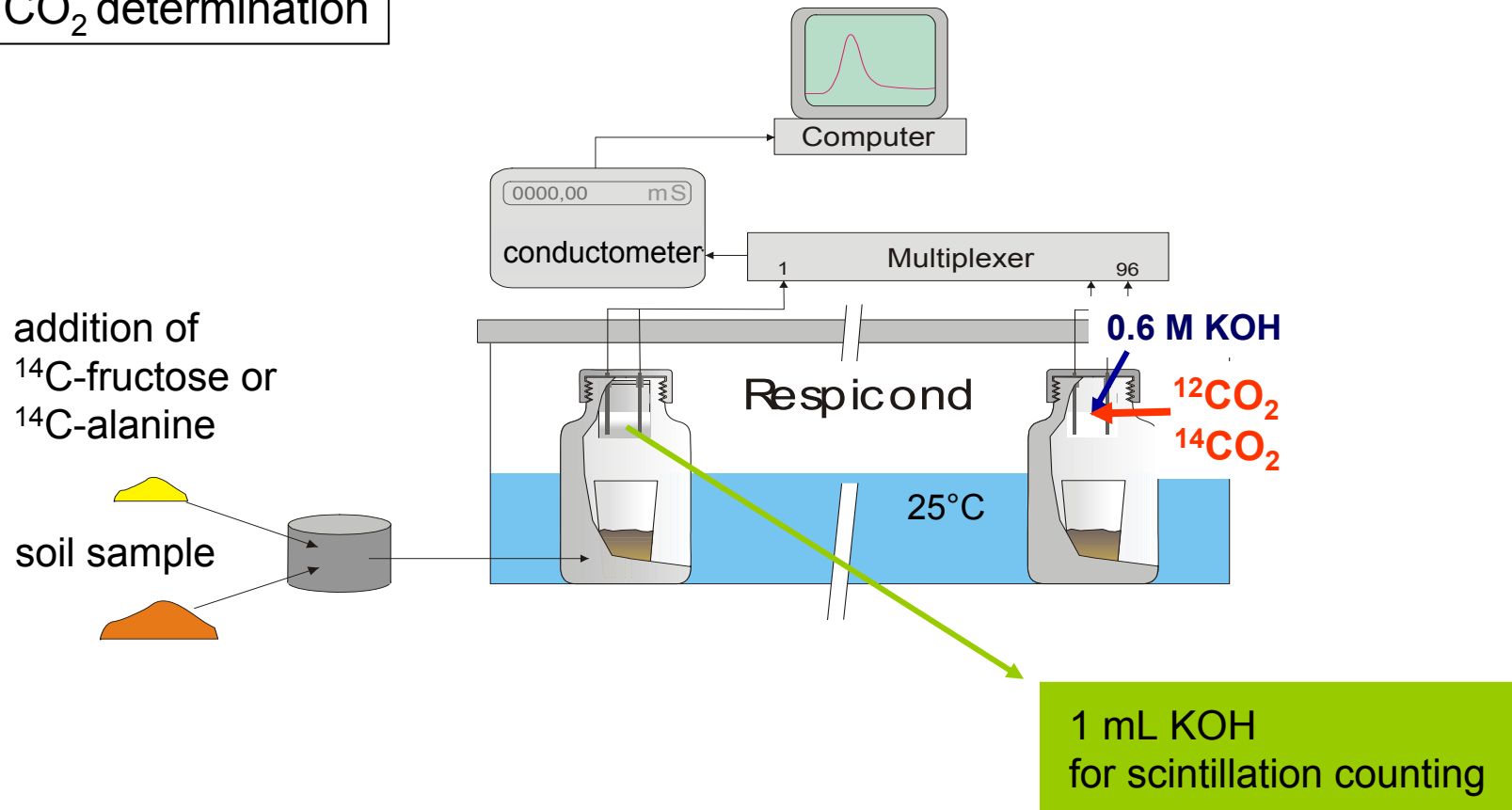
- (a) acceleration of SOM decomposition – positive priming effect
- (b) retardation of SOM decomposition – negative priming effect

modified after Kuzyakov et al. (2000)

Materials and Methods

Incubation of soil samples with additions of easily available organic substrates

CO₂ determination



Gosda, W. modified after Nordgren (1988)

Introduction

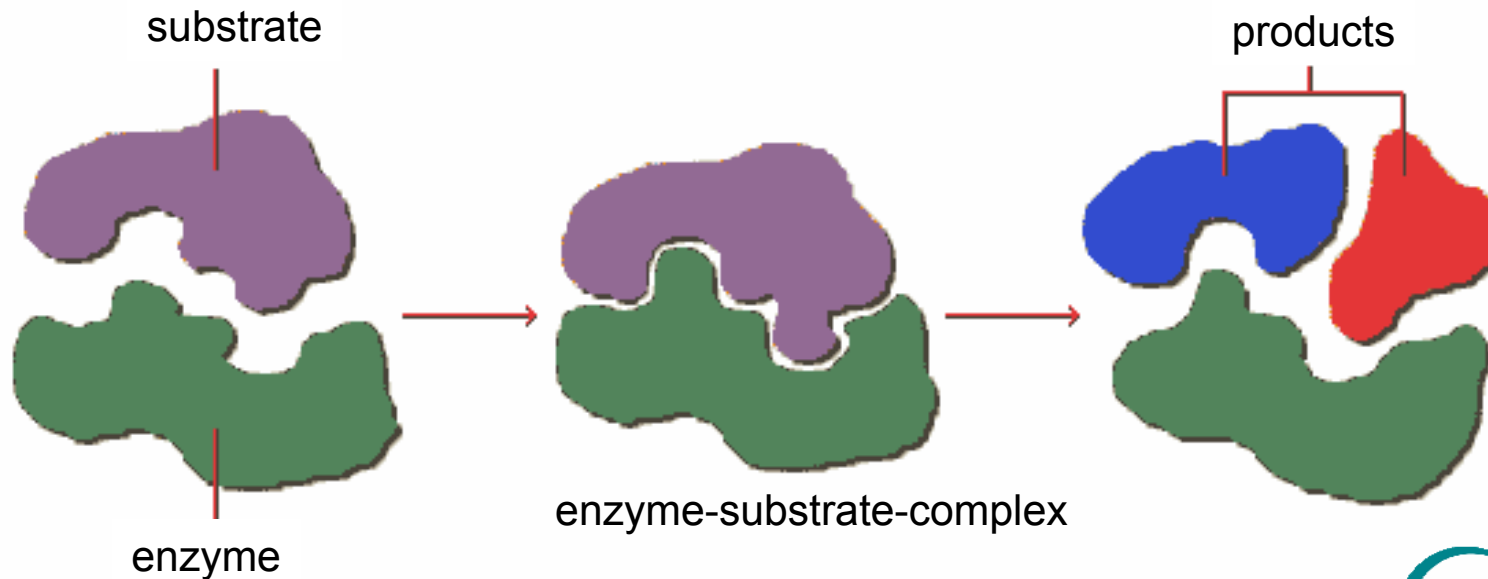
Treated wastewater is a source of **organic carbon**.

organic carbon = substrate for soil microorganisms

↳ **Effect on soil microbial communities and activities**

↳ **Hypothesis:** increased release of exoenzymes into the soil and alteration in mobilisation of nutrients

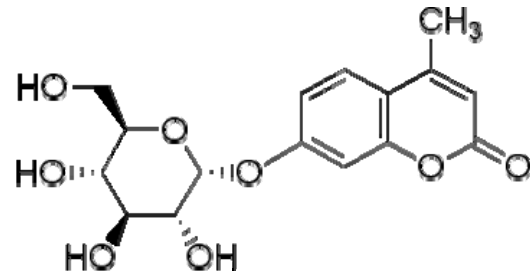
↳ ? Effects along soil profiles



Materials and Methods

A range of hydrolytic enzymes, involved in C, N and P cycles, were investigated using a fluorimetric microplate assay (after Marx et al. 2001).

**Measurements with the help of fluorescence substrates
(4-MUF = 4-Methylumbelliferon and AMC = 7-amino-4-methylcoumarin)**



4-MUF- α -D-Glycoside

α -D-Glycosidase \longrightarrow degradation of soluble sugars and starch

α -Glucosidase

β - Glucuronidase

β -Cellobiohydrolase

β - Xylosidase

β - Glucosidase

N-acetyl- β -glucosaminidase

Acid phosphatase

Leucin
aminopeptidase

Tyrosin
aminopeptidase

Arginin
aminopeptidase

Results

Soil microbiological parameter along 3 irrigated soil profiles - Bazra, Acco and Wadi Fara -

Soil depth [cm]	C _{mic}	C _{mic} /C _{org}	q CO ₂	acc. CO ₂	SOC _{min}	SOC _{min} alanine	SOC _{min} fructose	PE alanine	PE fructose
0-10	+	+ -		++	++ -	++ -	++ -	+ -	-
10-20	++	+ -		+++	+++	+	++ -	--	---
20-30		+	+	+	+ -	++	+ -		-
30-50	+ -	-		+++	+	---	--	(2)	- (2)
50-70	+ -	-			+ -	+ -	+		+
70-100	++ -	--	+ - -	+ -	-	-	--	(2)	- (2)

Bazra, Acco and Wadi Fara

	no difference between TWW and FW
---	TWW < FW (three sampling sites)
--	TWW < FW (two sampling sites)
-	TWW < FW (one sampling site)
+ - -	TWW < FW (two sampling sites) and TWW > FW (one sampling site)
+ -	TWW < FW (one sampling site) and TWW > FW (one sampling site)
++ -	TWW < FW (one sampling site) and TWW > FW (two sampling sites)
+	TWW > FW (one sampling site)
++	TWW > FW (two sampling sites)
+++	TWW > FW (three sampling sites)

Student t-Test p<0.05

(2) - only two of three sampling sites showed significant differences between control and treated sample, therefore only these values were calculated

Results

Soil microbiological parameter along 3 irrigated soil profiles - Bazra, Acco and Wadi Fara -

Soil depth [cm]	α -glu	β -Xyl	N-acet	β -glucoro	β -cello	β -glu	pho	Leu	Tyr	Arg
0-10		+	++	+	++	++	+ -	++	+++	++
10-20	+	+		+	+	+	+	++	+	+
20-30		+		+	++	++	++	++	+	++
30-50	+ -	+ -	+ -		+	+	++	+	+ -	++
50-70		+ -	+ -	-		+ -	+	++ -	-	+ -
70-100	-	-	-		-	-	-	-	-	+ -

Bazra, Acco and Wadi Fara

	no difference between TWW and FW
---	TWW < FW (three sampling sites)
--	TWW < FW (two sampling sites)
-	TWW < FW (one sampling site)
+ - -	TWW < FW (two sampling sites) and TWW > FW (one sampling site)
+ -	TWW < FW (one sampling site) and TWW > FW (one sampling site)
++ -	TWW < FW (one sampling site) and TWW > FW (two sampling sites)
+	TWW > FW (one sampling site)
++	TWW > FW (two sampling sites)
+++	TWW > FW (three sampling sites)

Student t-Test $p < 0.05$

(2) - only two of three sampling sites showed significant differences between control and treated sample, therefore only these values were calculated

α -glu = α -glucosidase; β -Xyl = β -Xylosidase; N-acet = N-acety- β -glucosamidase, pho = acid phosphatase; β -glucoro = β -glucosidase; β -cello = β -cellobiohydrolase; β -glu = β -glucosidase; Leu = Leucin aminopeptidase; Tyr = Tyrosin aminopeptidase; Arg = Argin aminopeptidase

Method: Marx et al. 2001

Results

Soil microbiological parameter along 3 irrigated soil profiles - Bazra, Acco and Wadi Fara -

Soil depth [cm]	α -glu	β -Xyl	N-acet	β -glucoro	β -cello	β -glu	pho	Leu	Tyr	Arg
0-10		+	++	+	++	++	+ -	++	+++	++
10-20	+	+		+	+	+	+	++	+	+
20-30		+		+	++	++	++	++	+	++
30-50	+ -	+ -	+ -		+	+	++	+	+ -	++
50-70		+								
70-100	-	-								

Changes in SOC- quality due to continuously priming in the field by addition of TWW – long-term effect

Bazra, Acco and Wadi Fara

	no difference
---	TWW < FW (three sampling sites)
--	TWW < FW (two sampling sites)
-	TWW < FW (one sampling site)
+ - -	TWW < FW (two sampling sites) and TWW > FW (one sampling site)
+ -	TWW < FW (one sampling site) and TWW > FW (one sampling site)
++ -	TWW < FW (one sampling site) and TWW > FW (two sampling sites)
+	TWW > FW (one sampling site)
++	TWW > FW (two sampling sites)
+++	TWW > FW (three sampling sites)

Student t-Test p<0.05

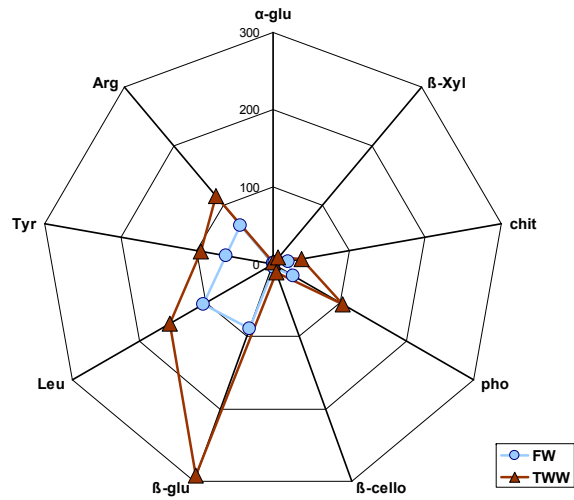
if three sampling sites showed significant differences between control and treated sample, therefore only these values were calculated

α -glu = α -glucosidase; β -Xyl = β -Xylosidase; N-acet = N-acety- β -glucosamidase, pho = acid phosphatase; β -glucoro = β -glucosidase; β -cello = β -cellobiohydrolase; β -glu = β -glucosidase; Leu = Leucin aminopeptidase; Tyr = Tyrosin aminopeptidase; Arg = Argin aminopeptidase

Method: Marx et al. 2001

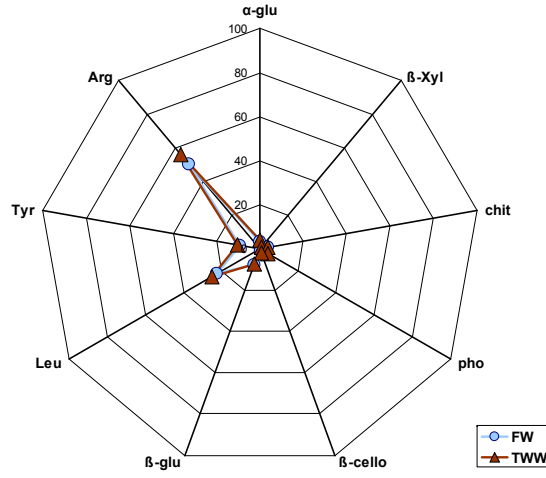
BAZRA – enzyme activities

0-10 cm



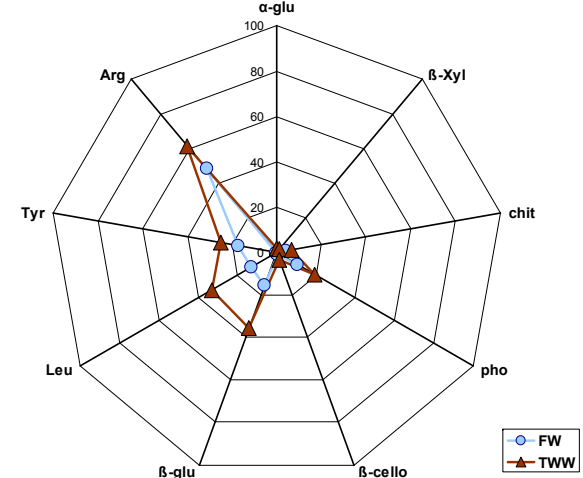
max: 291.6 nmol g⁻¹ h⁻¹

10-20 cm



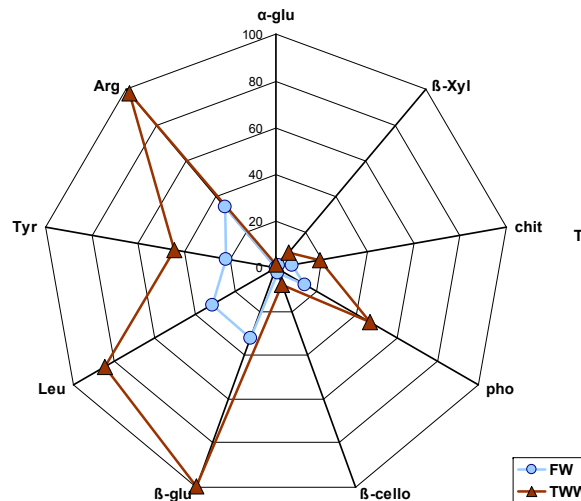
max: 55.7 nmol g⁻¹ h⁻¹

20-30 cm



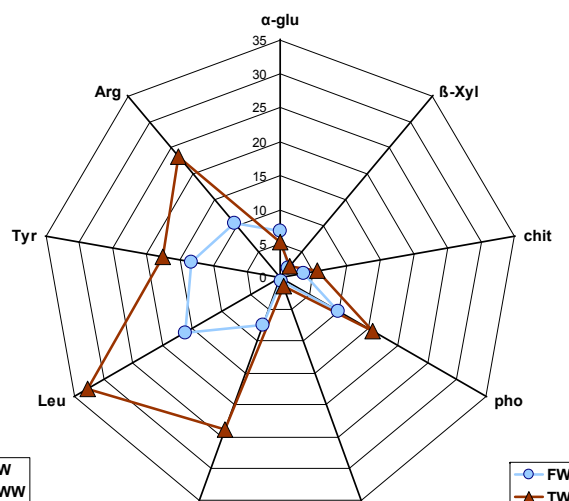
max: 61.2 nmol g⁻¹ h⁻¹

30-50 cm



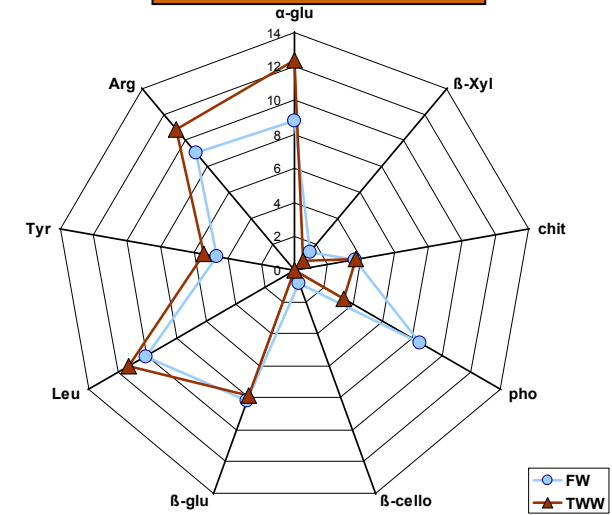
max: 99.6 nmol g⁻¹ h⁻¹

50-70 cm



max: 32.6 nmol g⁻¹ h⁻¹

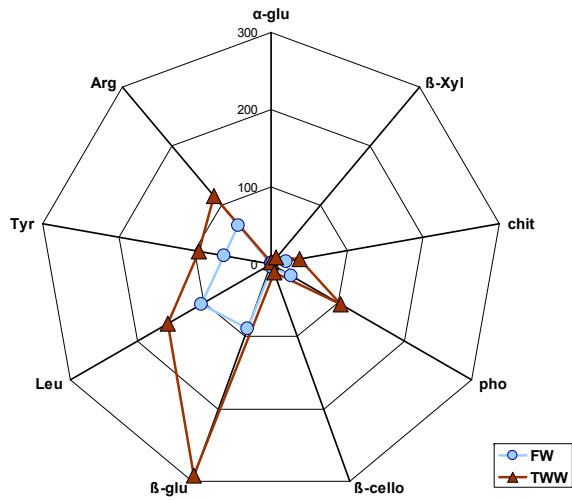
70-100 cm



max: 12.4 nmol g⁻¹ h⁻¹

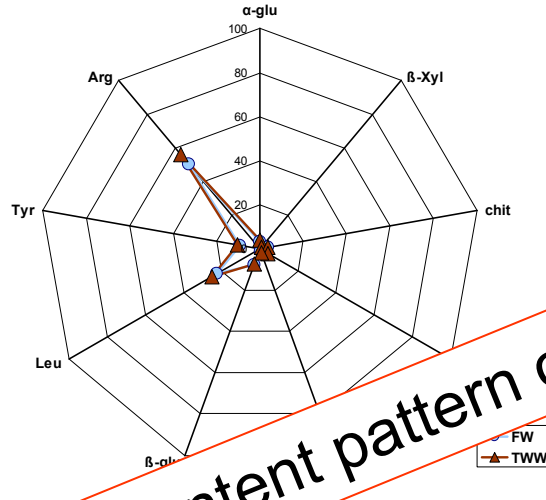
BAZRA – enzyme activities

0-10 cm



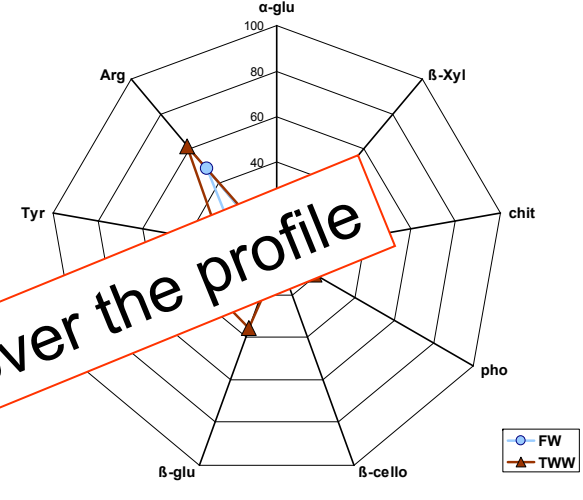
max: 291.6 nmol g⁻¹ h⁻¹

10-20 cm



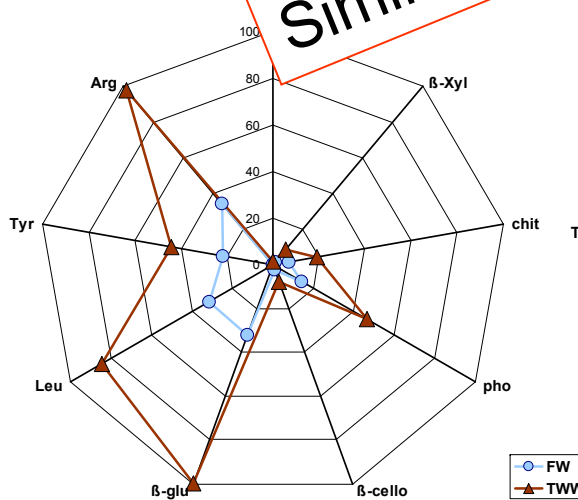
max: 77 nmol g⁻¹ h⁻¹

20-30 cm



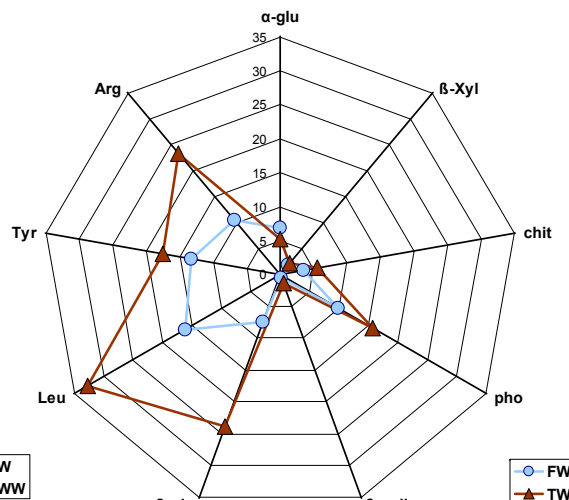
max: 61.2 nmol g⁻¹ h⁻¹

30-50 cm



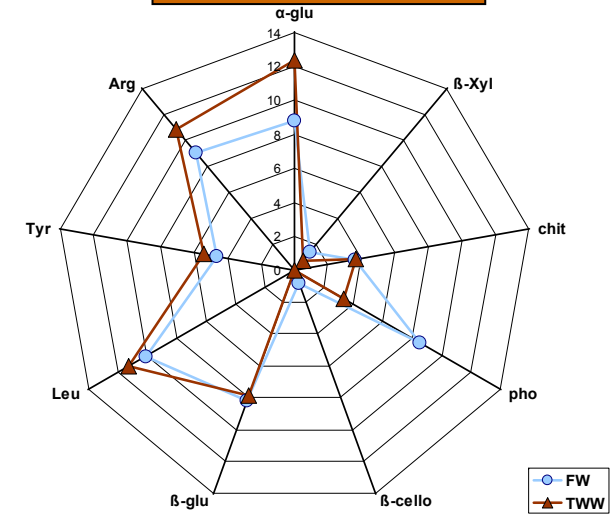
max: 99.6 nmol g⁻¹ h⁻¹

50-70 cm



max: 32.6 nmol g⁻¹ h⁻¹

70-100 cm



max: 12.4 nmol g⁻¹ h⁻¹

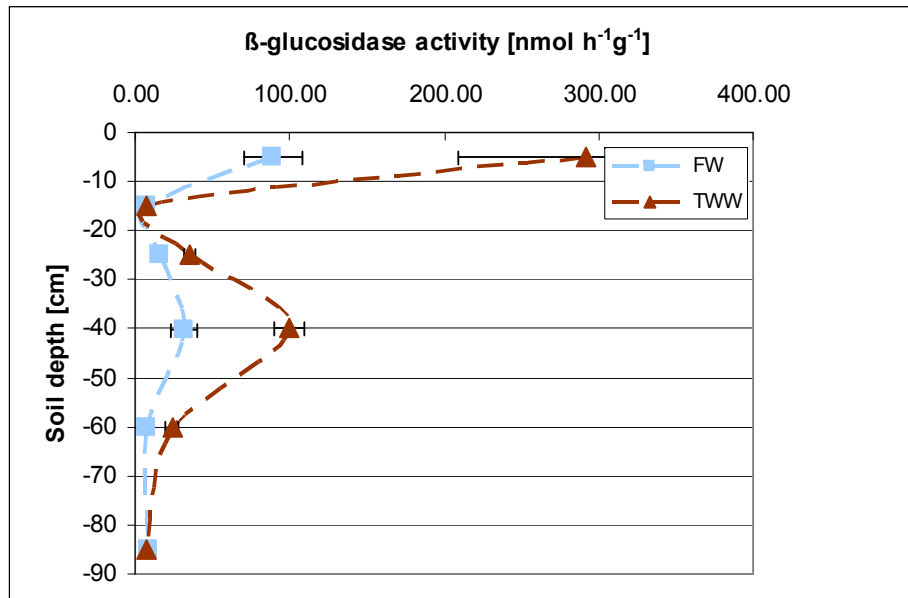
Similar enzyme content pattern over the profile

Results

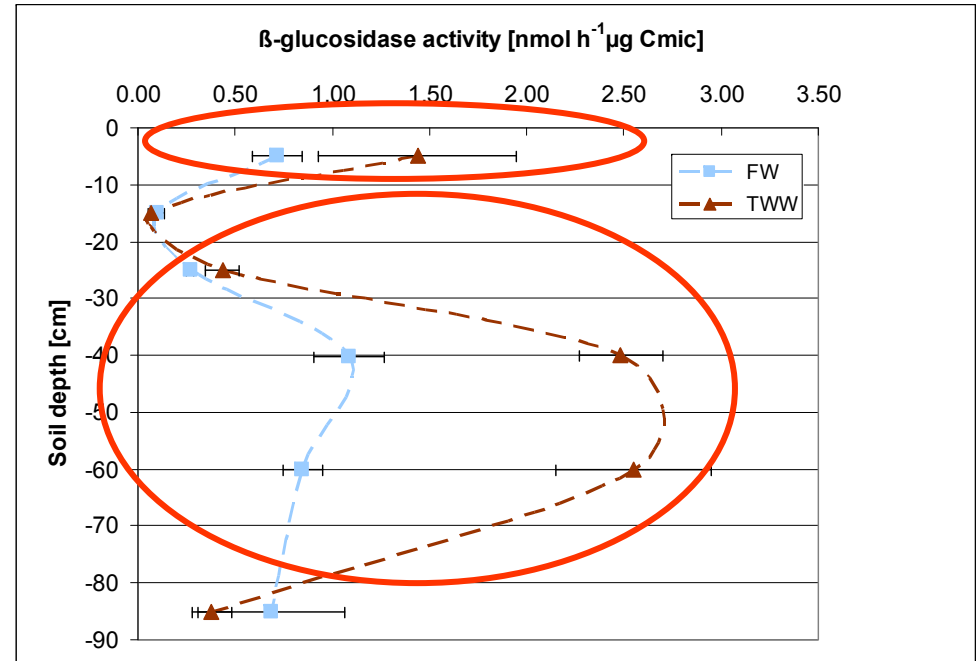
Bazra – soil enzyme profiles

β -glucosidase

nmol h⁻¹ g⁻¹ dry soil



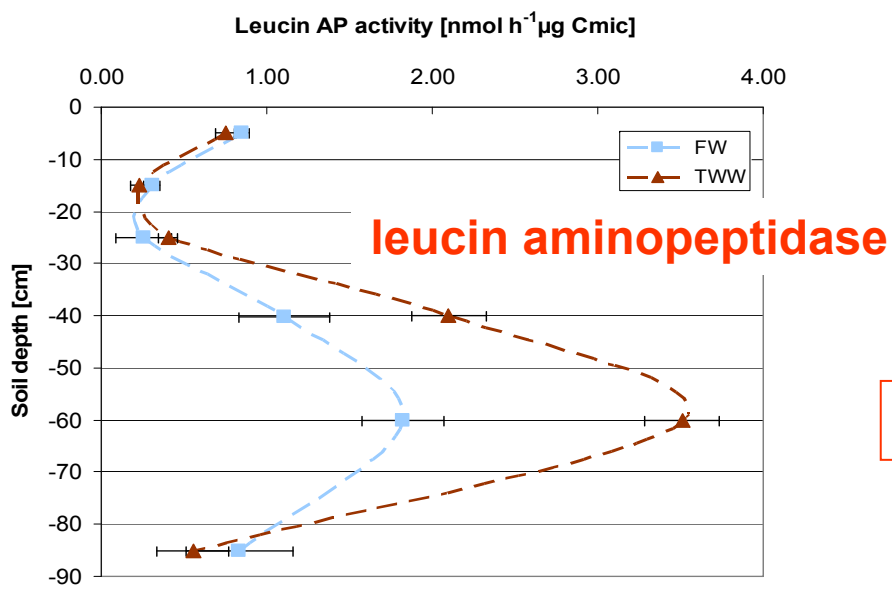
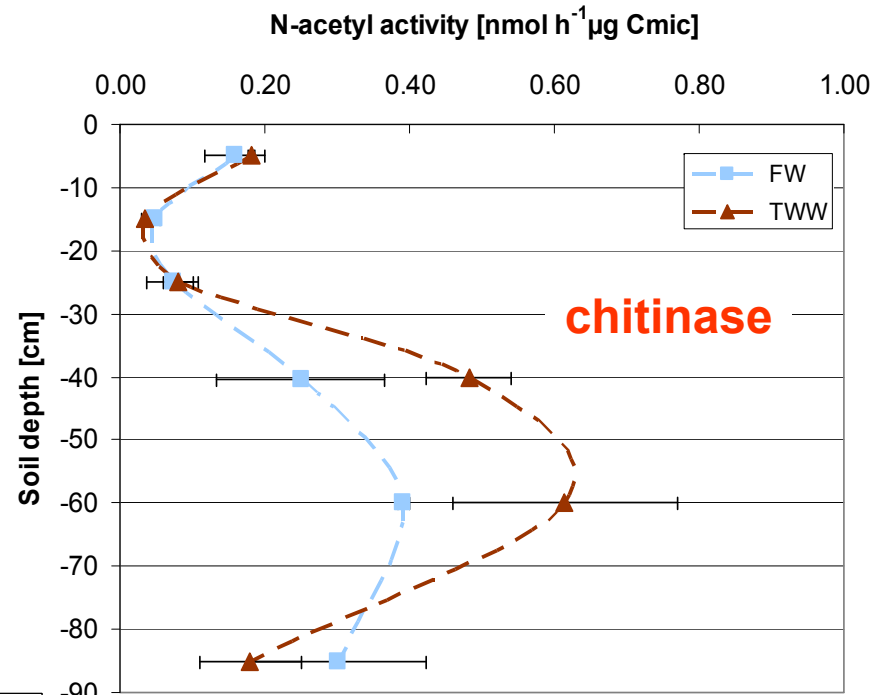
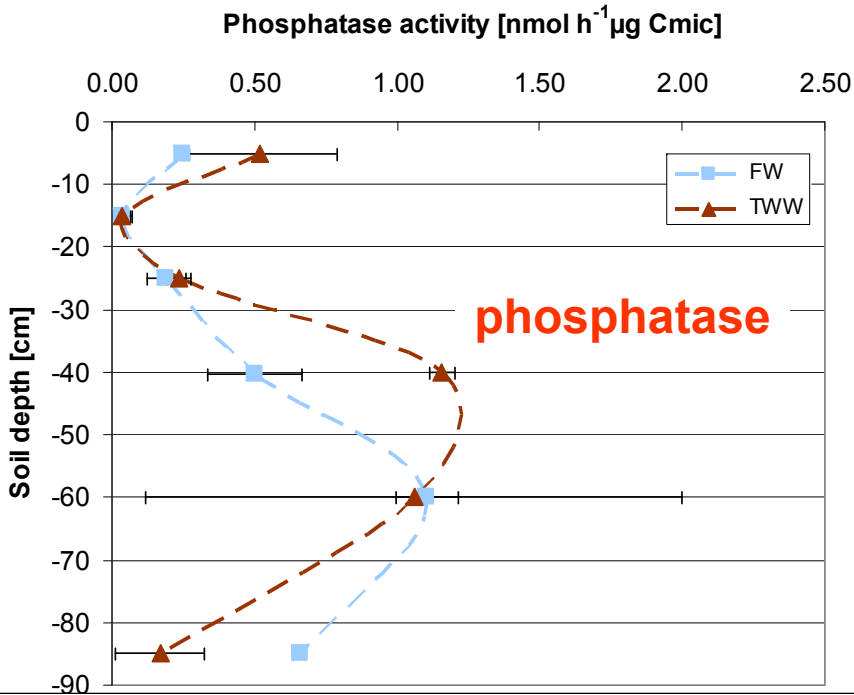
nmol h⁻¹ μ g⁻¹ C_{mic}



- ➔ clear differences between FW and TWW
- ➔ activity increase with soil depth
- ➔ high correlation with microbial biomass carbon

Results & Discussion

Bazra – soil enzyme profiles



nmol h⁻¹ μg⁻¹ C_{mic}

➔ similar pattern like β-glucosidase

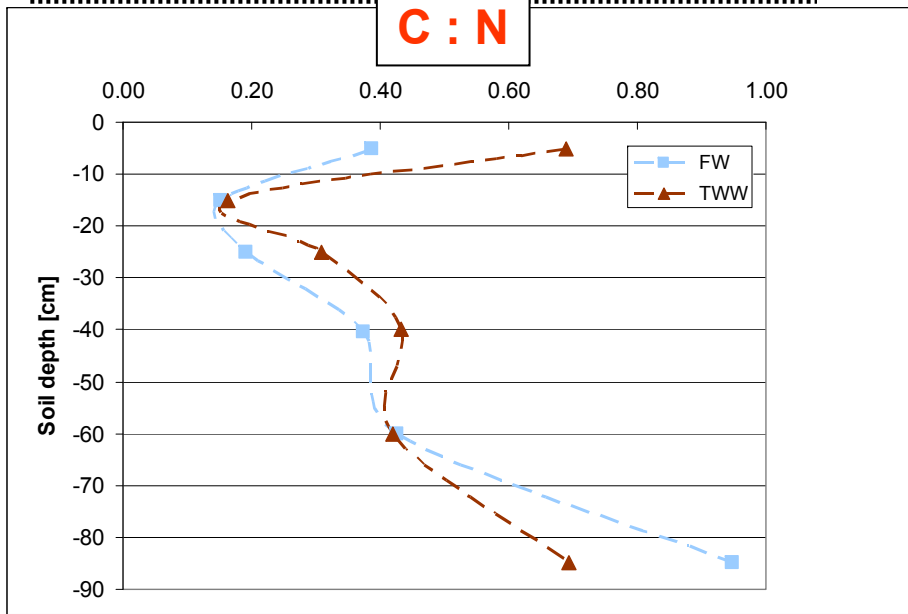
➔ activity increase with soil depth

maybe due to tree roots or shift in texture

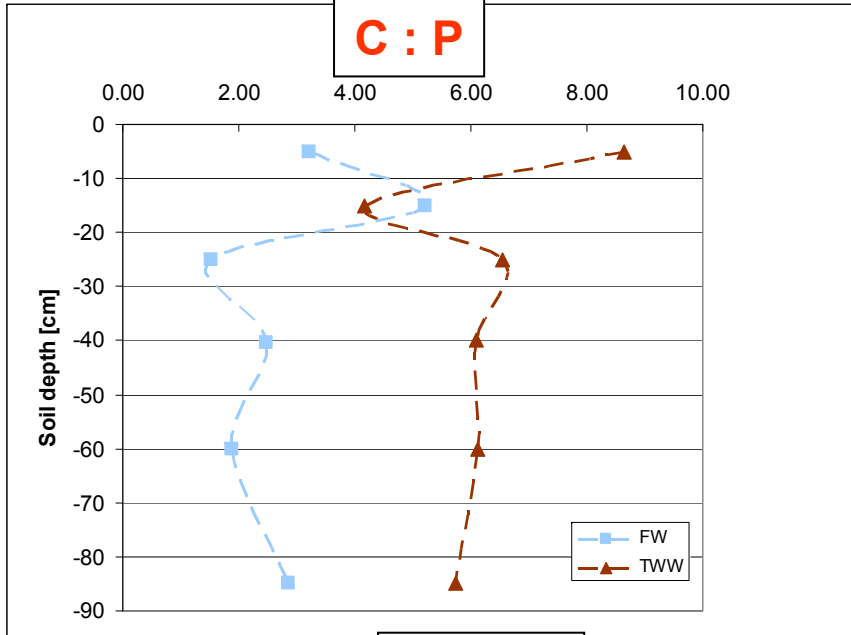
Increased amounts of tree roots under TWW

Results

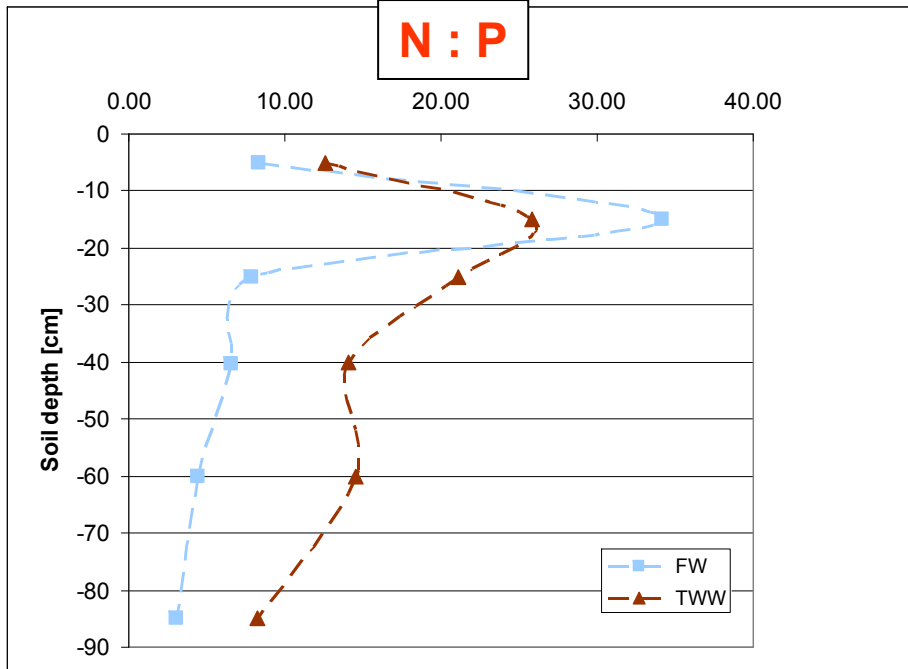
C : N



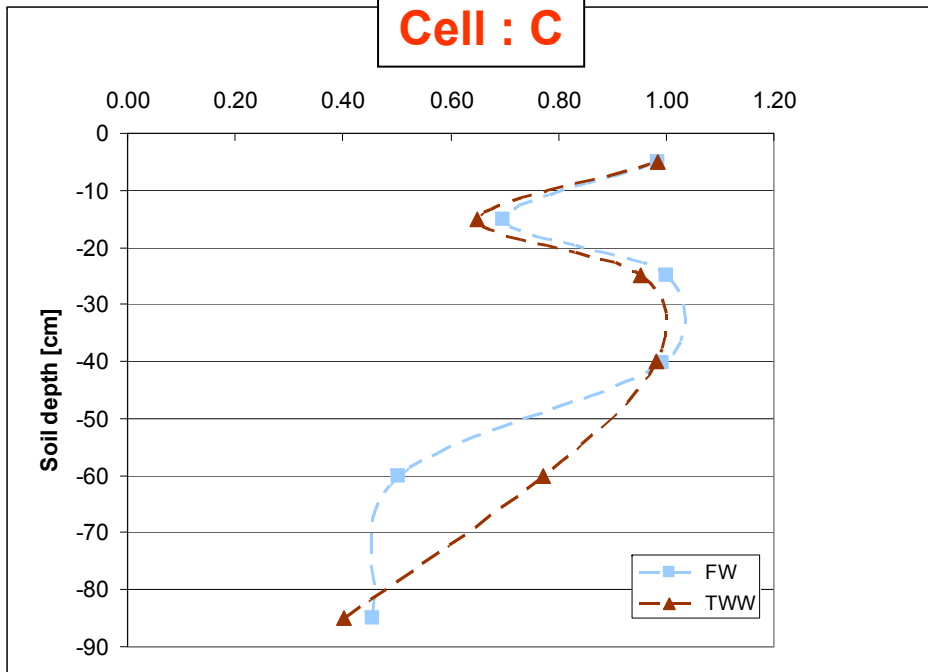
C : P



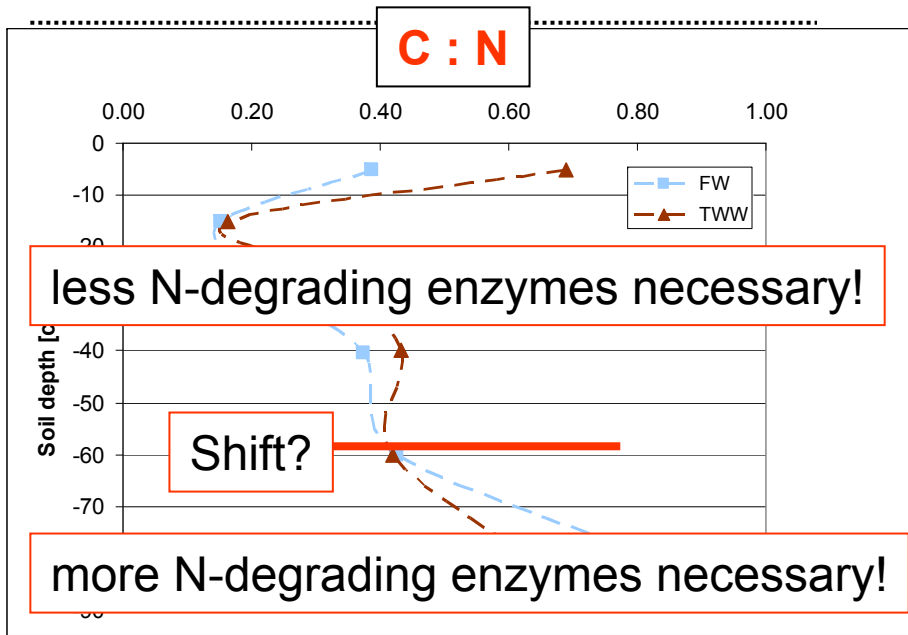
N : P



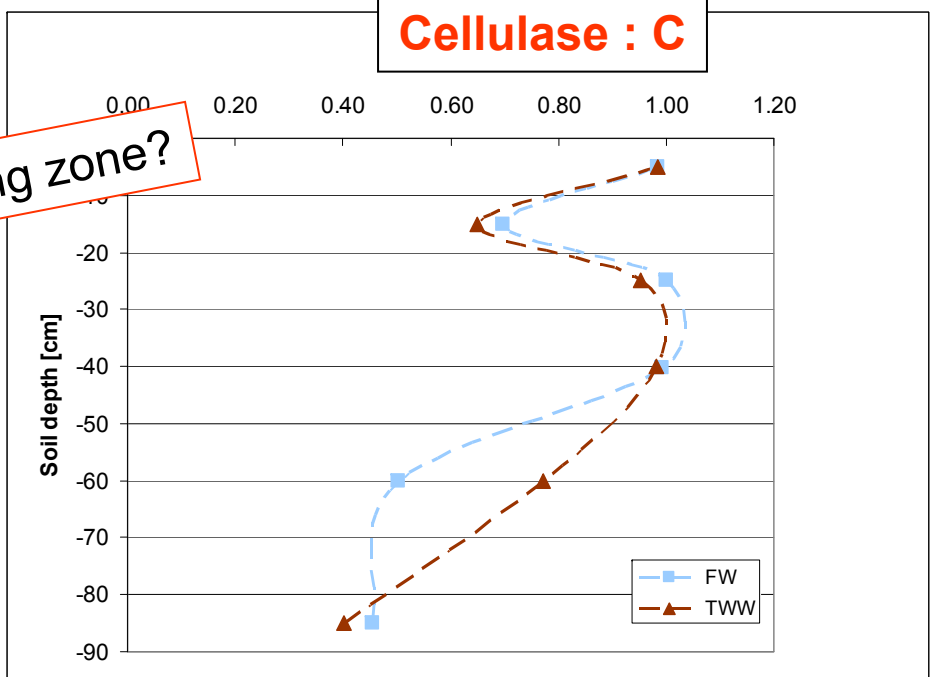
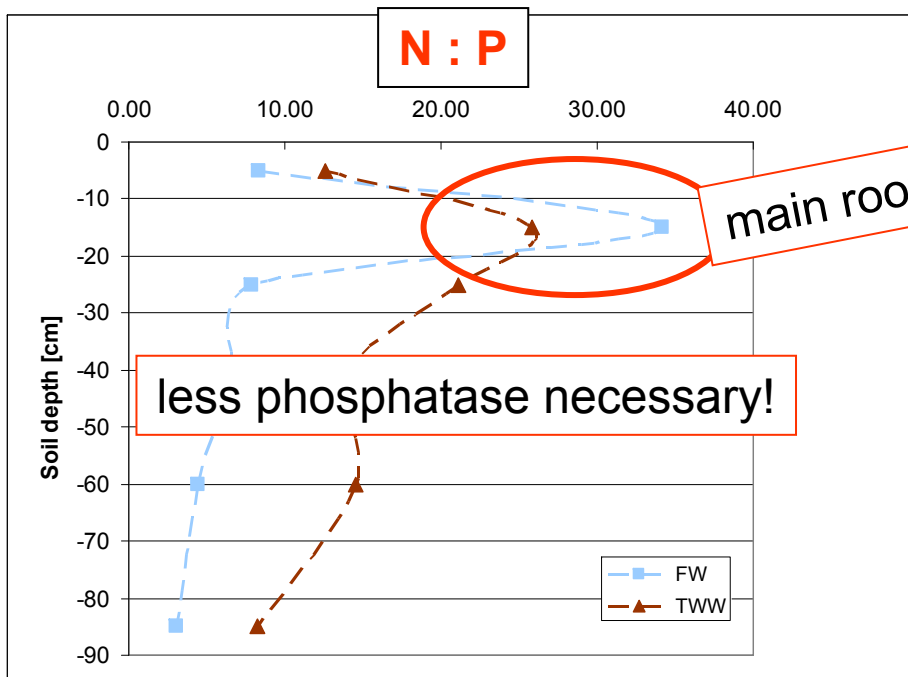
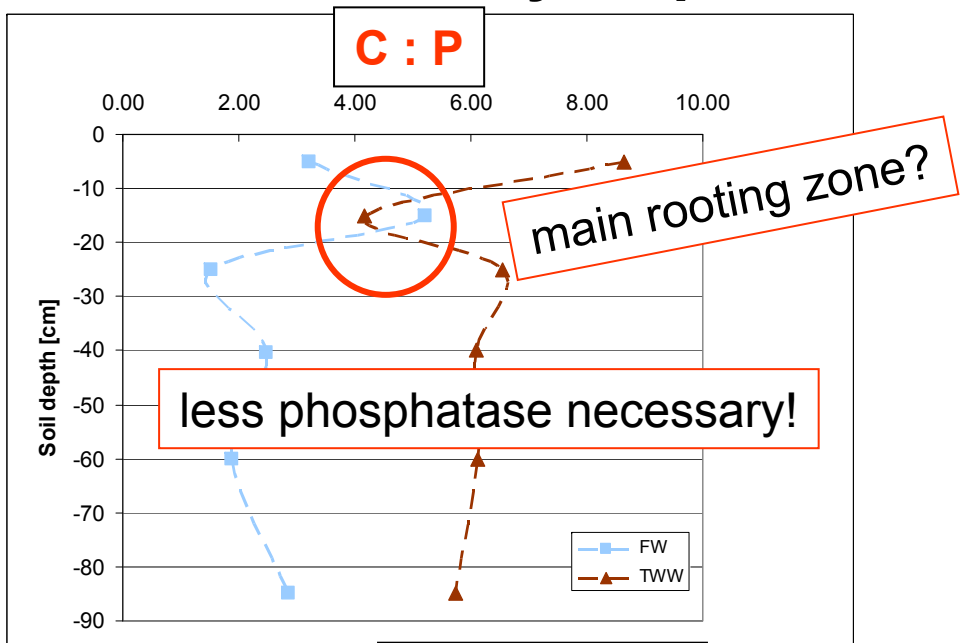
Cell : C



Results

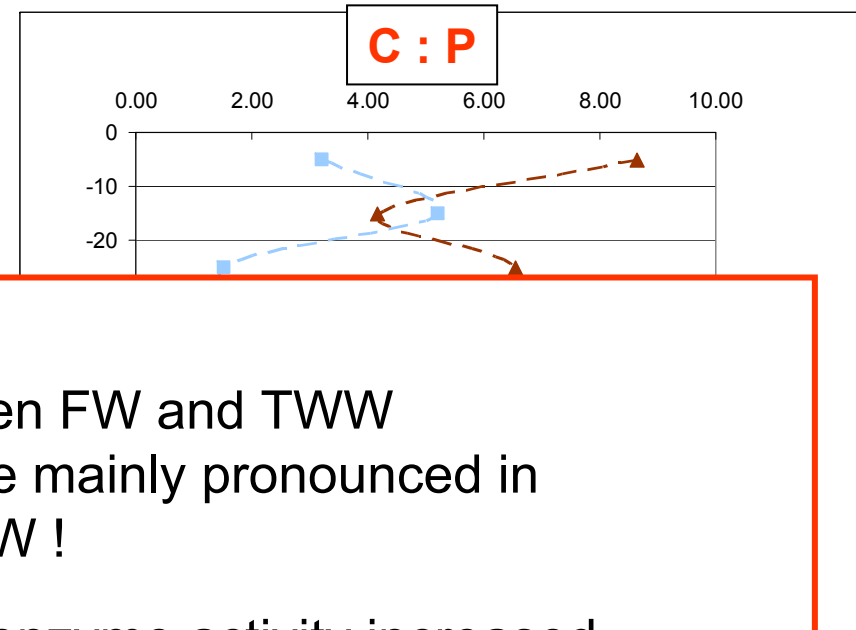
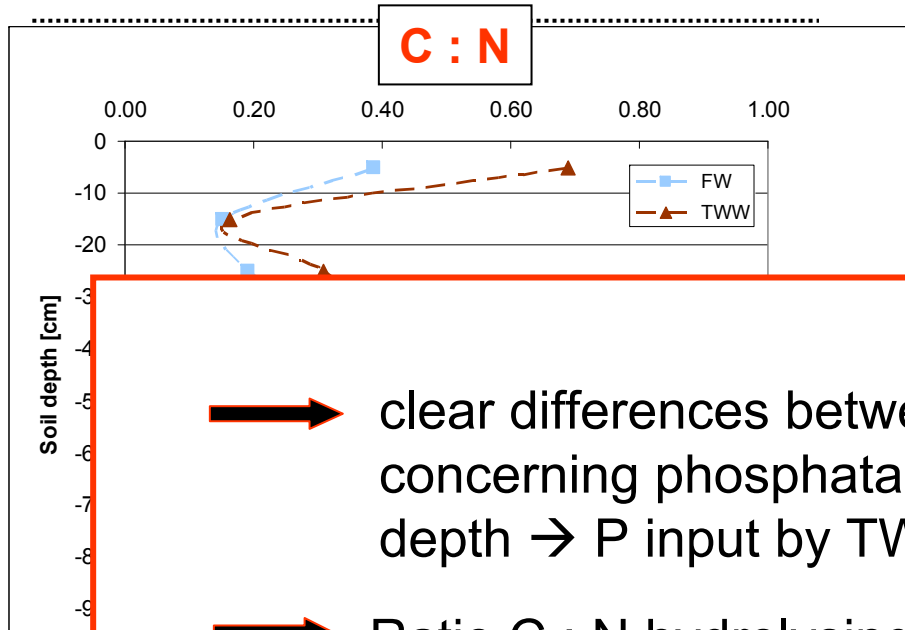


Bazra – soil enzyme profiles

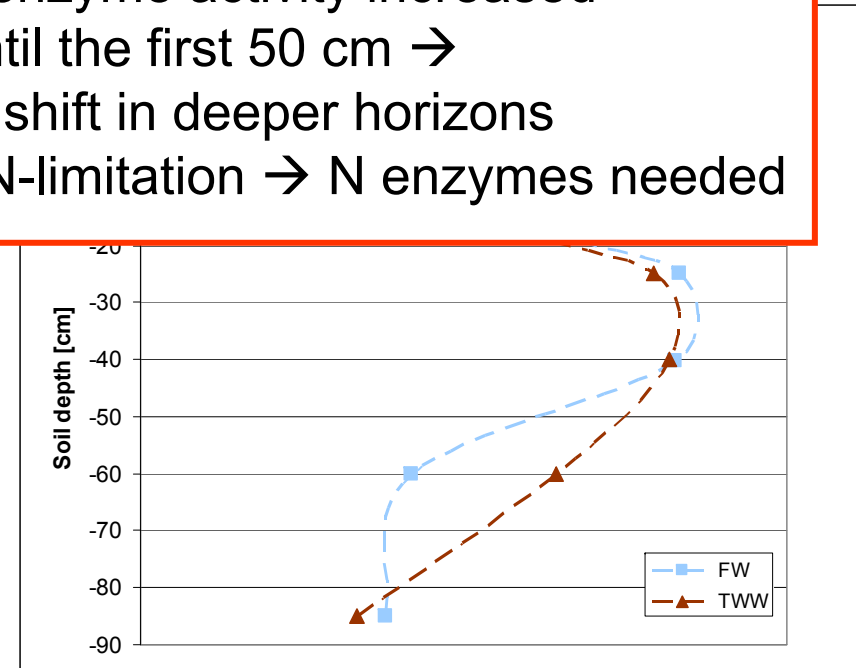
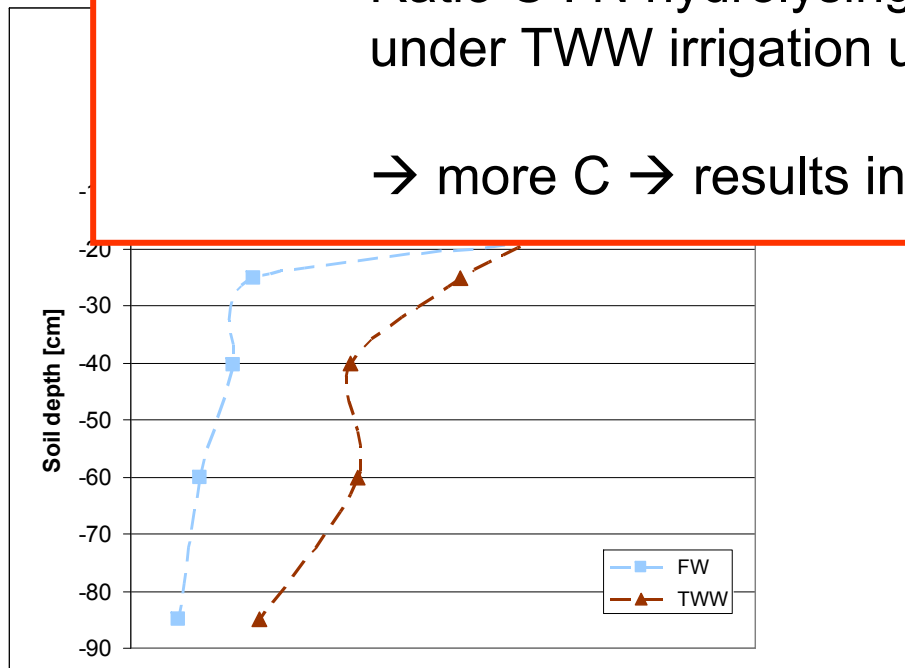


Results

Bazra – soil enzyme profiles

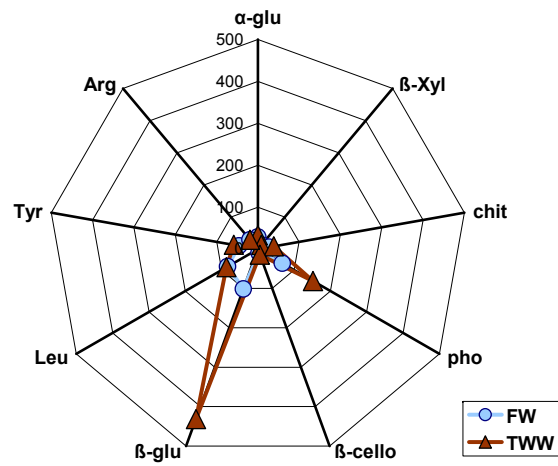


- ➔ clear differences between FW and TWW concerning phosphatase mainly pronounced in depth → P input by TWW !
- ➔ Ratio C : N hydrolysing enzyme activity increased under TWW irrigation until the first 50 cm → shift in deeper horizons → more C → results in N-limitation → N enzymes needed



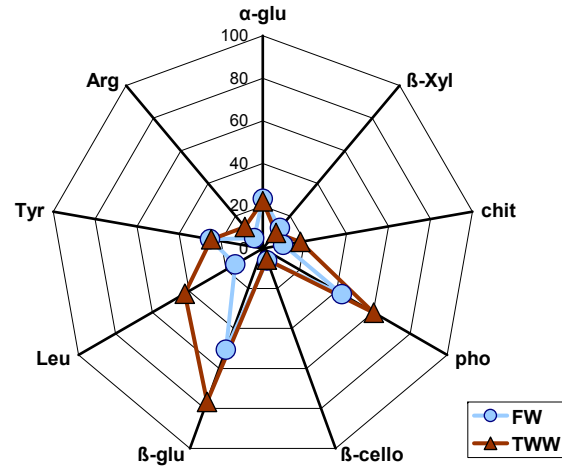
Acco – enzyme activities

0-10 cm



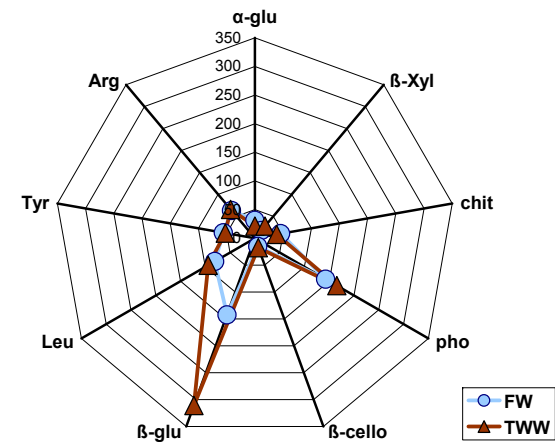
max: 431.4 nmol g⁻¹ h⁻¹

10-20 cm



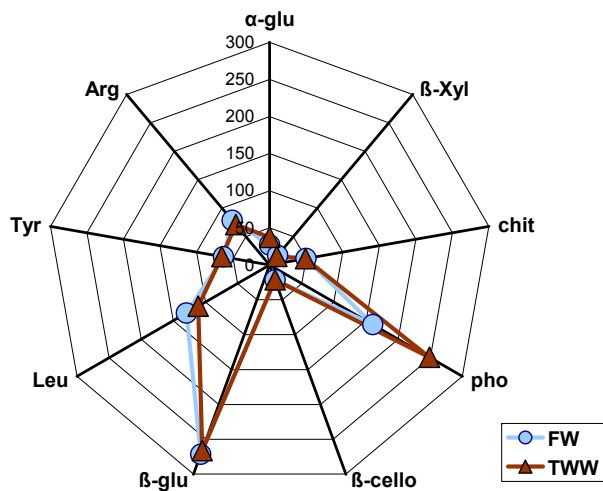
max: 76.7 nmol g⁻¹ h⁻¹

20-30 cm



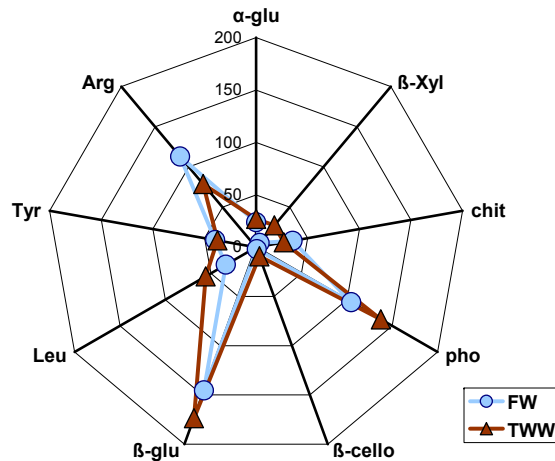
max: 310.6 nmol g⁻¹ h⁻¹

30-50 cm



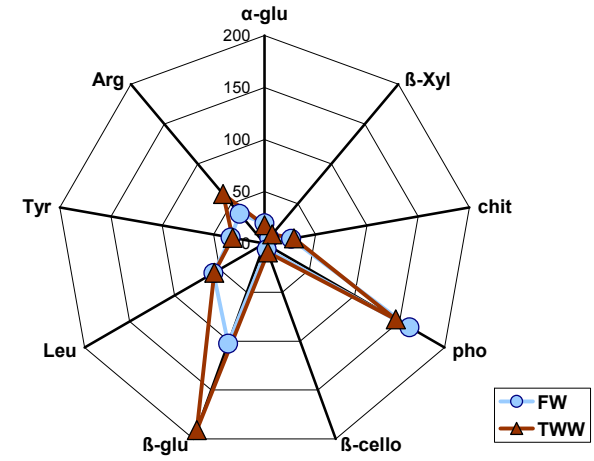
max: 271.3 nmol g⁻¹ h⁻¹

50-70 cm



max: 173.6 nmol g⁻¹ h⁻¹

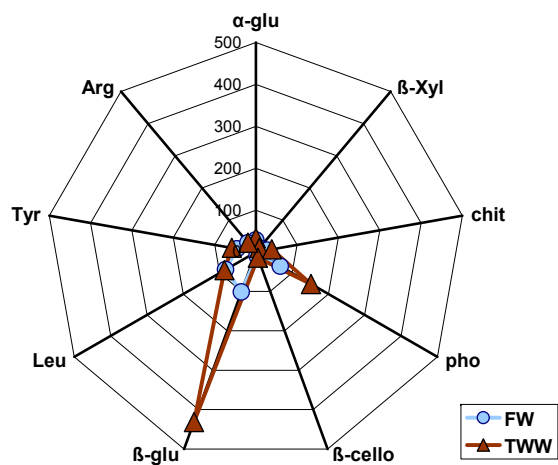
70-100 cm



max: 190.8 nmol g⁻¹ h⁻¹

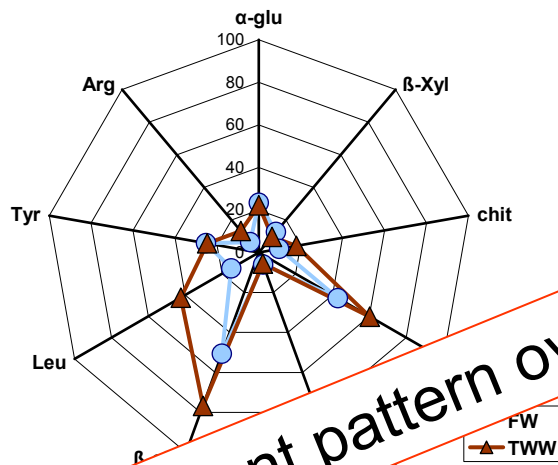
Acco – enzyme activities

0-10 cm



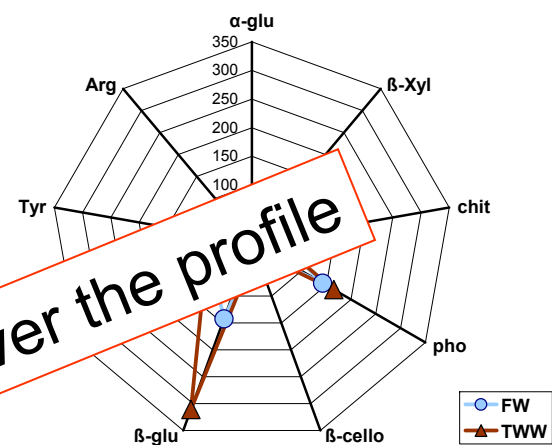
max: 431.4 nmol g⁻¹ h⁻¹

10-20 cm



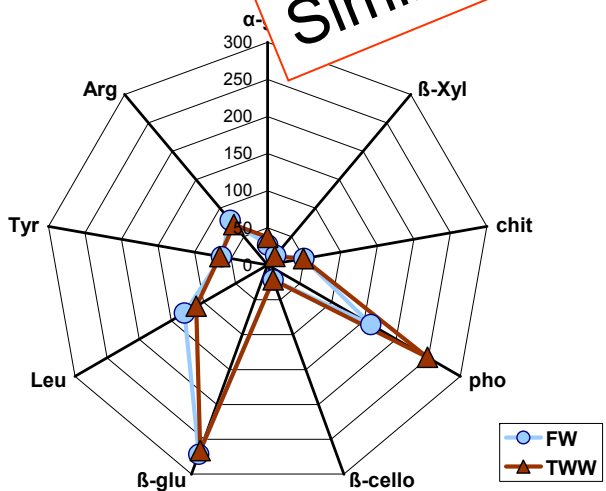
max: 100 nmol g⁻¹ h⁻¹

20-30 cm



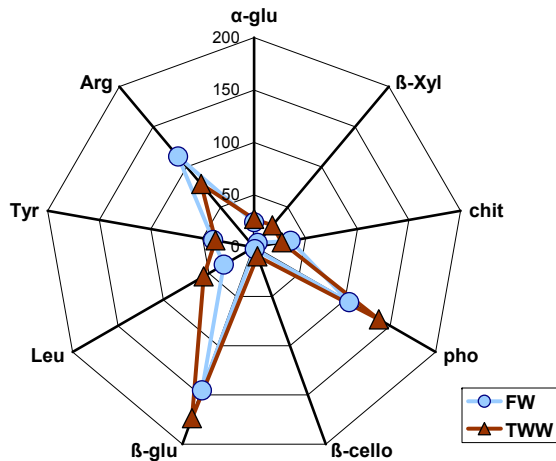
max: 310.6 nmol g⁻¹ h⁻¹

30-50 cm



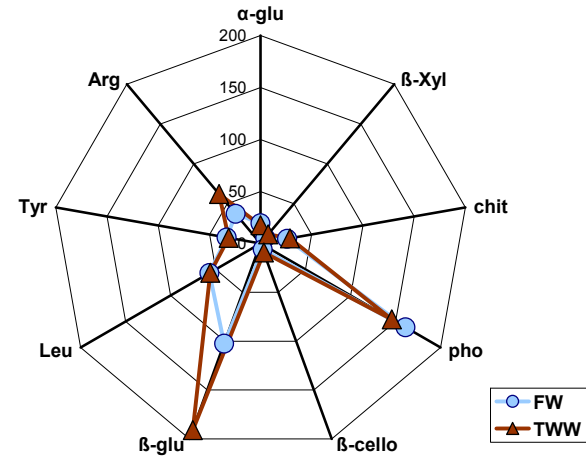
max: 271.3 nmol g⁻¹ h⁻¹

50-70 cm



max: 173.6 nmol g⁻¹ h⁻¹

70-100 cm



max: 190.8 nmol g⁻¹ h⁻¹

Similar enzyme content pattern over the profile

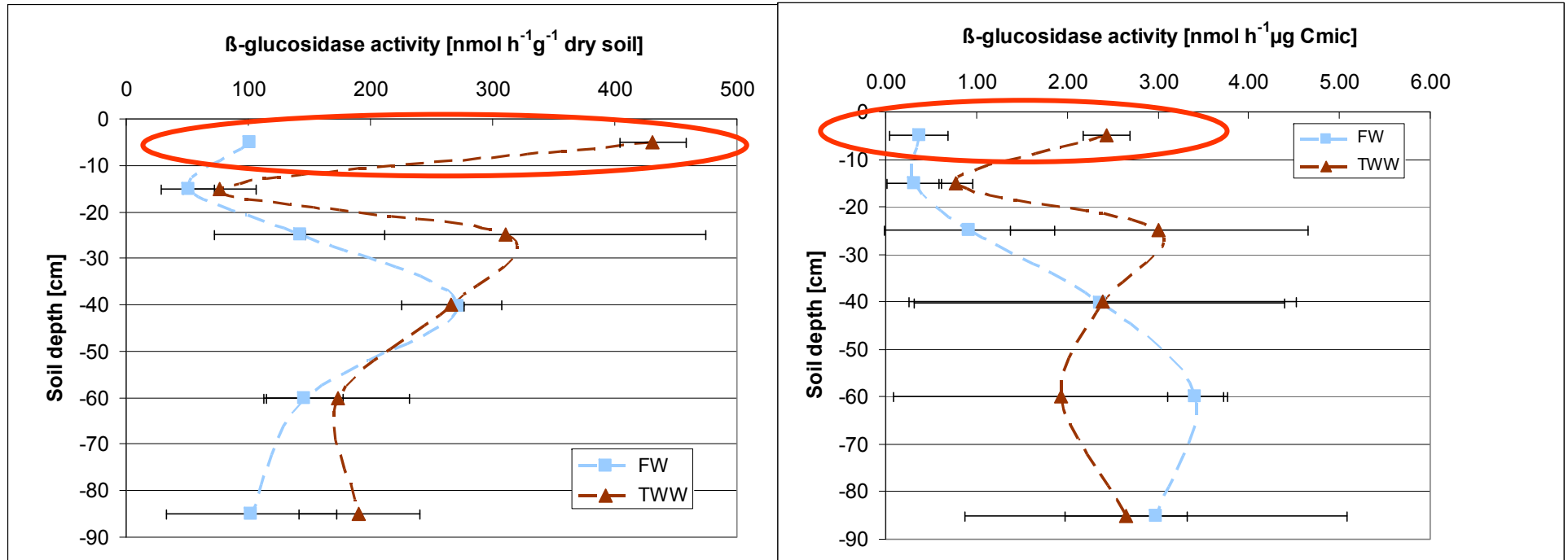
Results

Acco – soil enzyme profiles

β -glucosidase

nmol h⁻¹ g⁻¹ dry soil

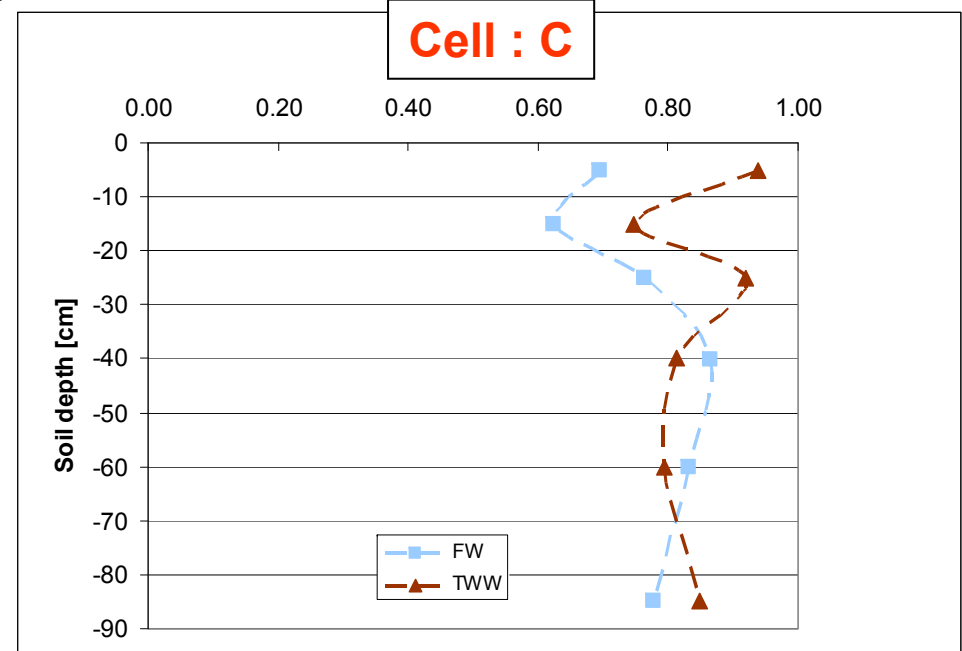
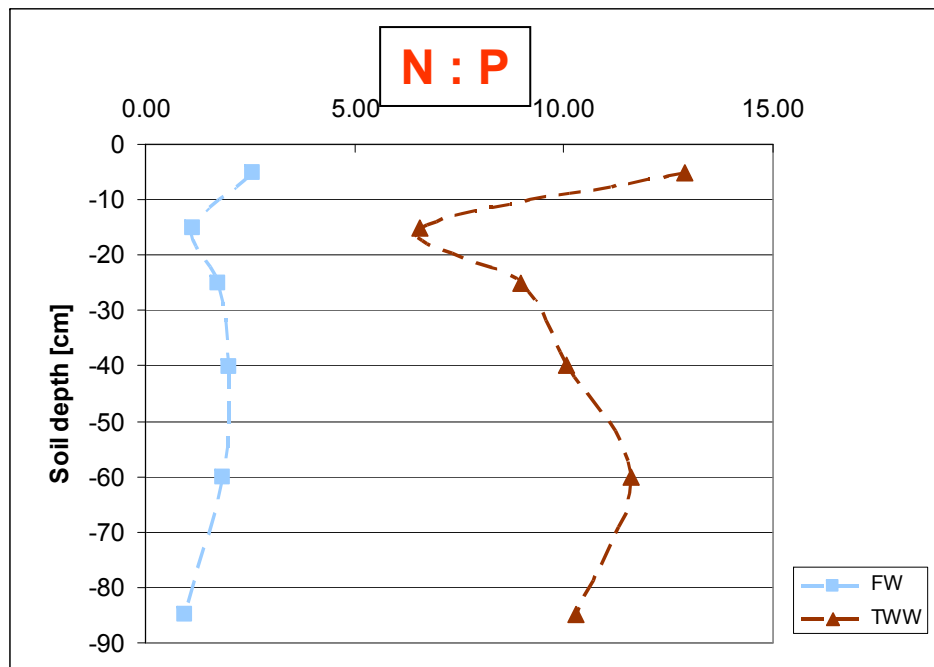
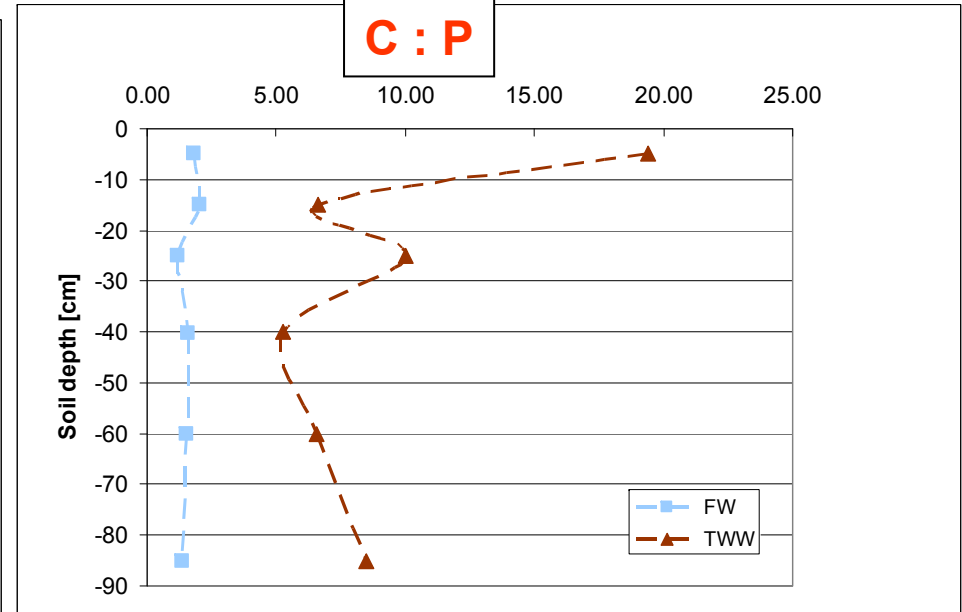
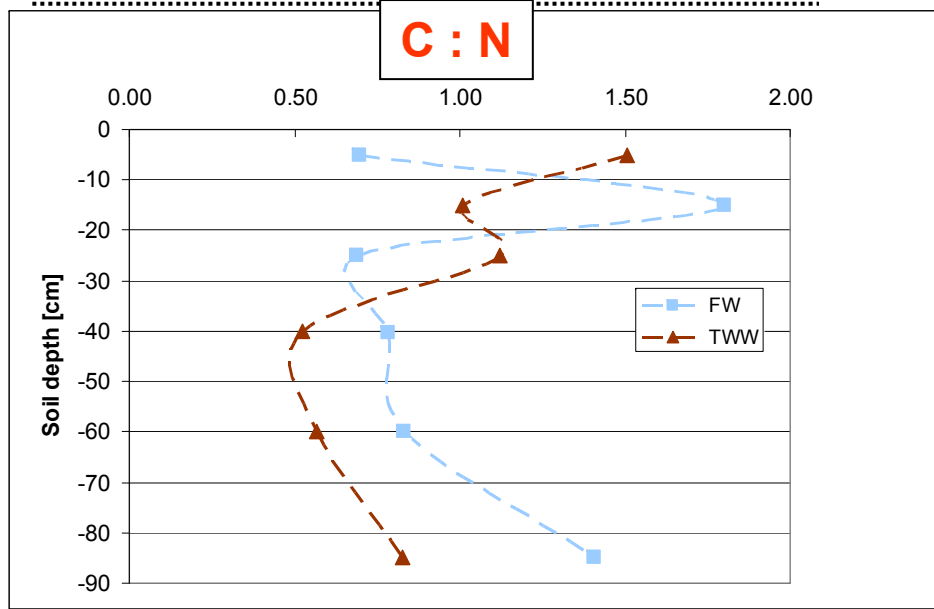
nmol h⁻¹ μ g⁻¹ C_{mic}



- ➡ clear differences between FW and TWW only in topsoil
- ➡ activity increase with soil depth only under FW irrigation
- ➡ Distribution along the profile reflects the transportation of carbon

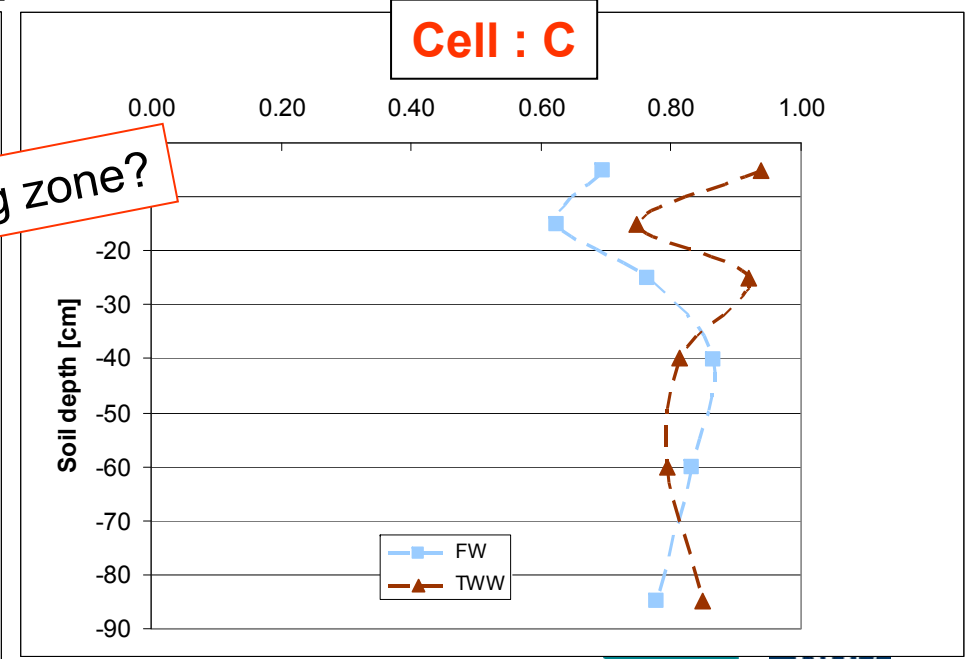
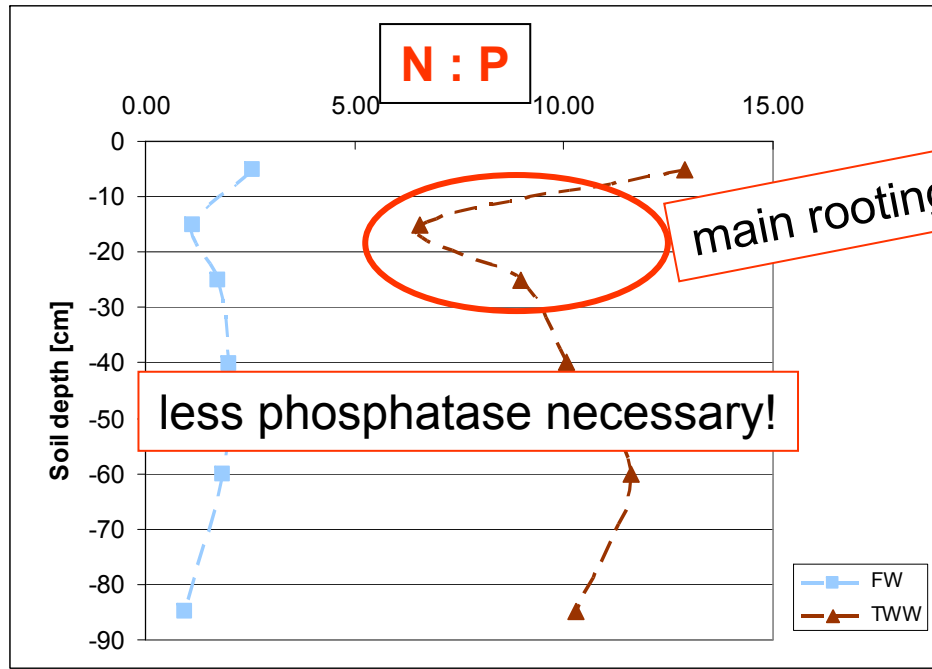
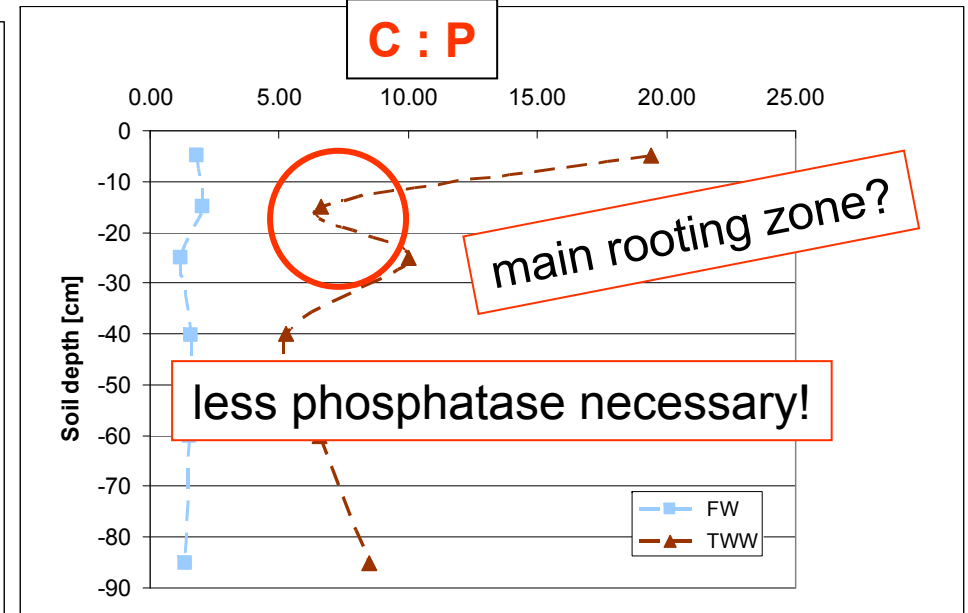
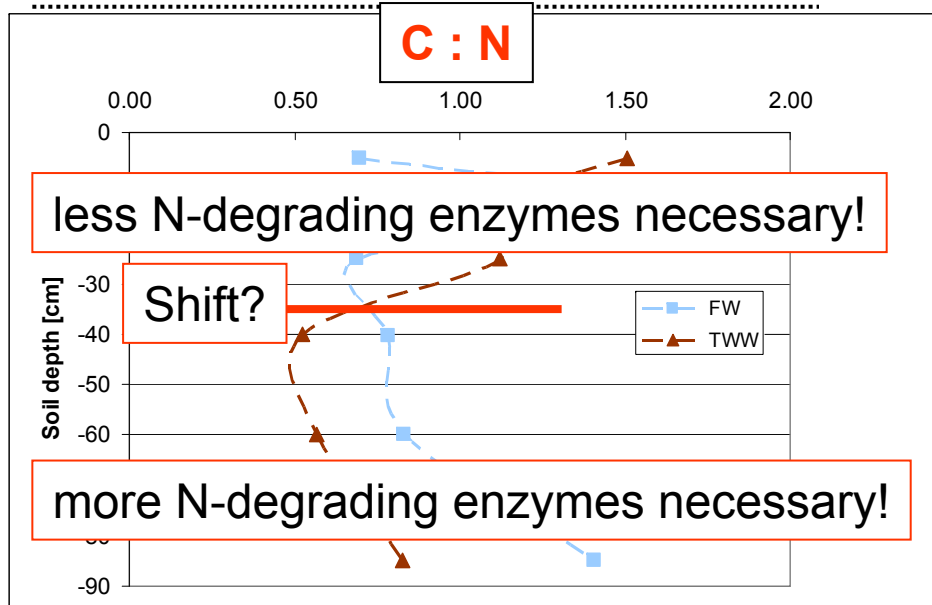
Results & Discussion

Acco – soil enzyme profiles



Results & Discussion

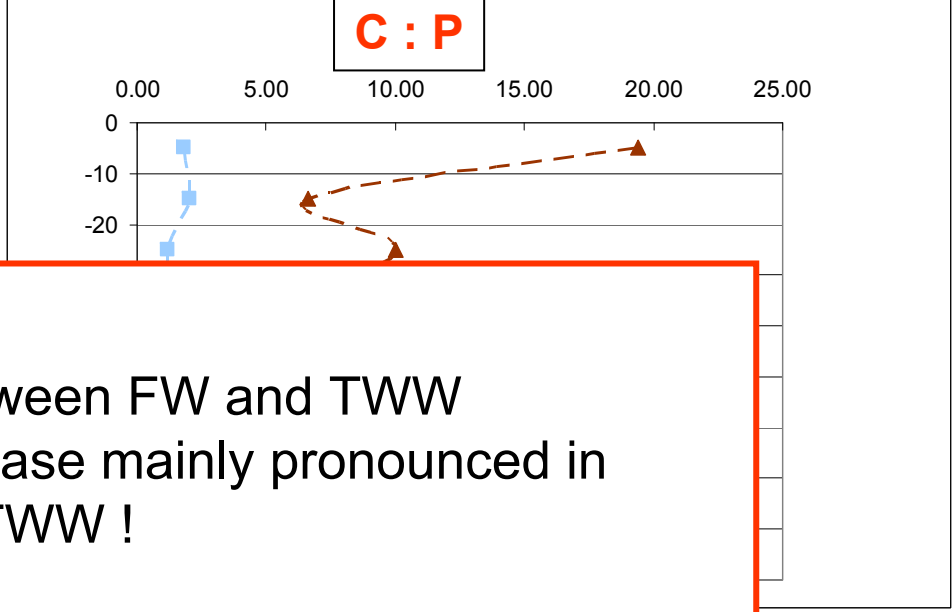
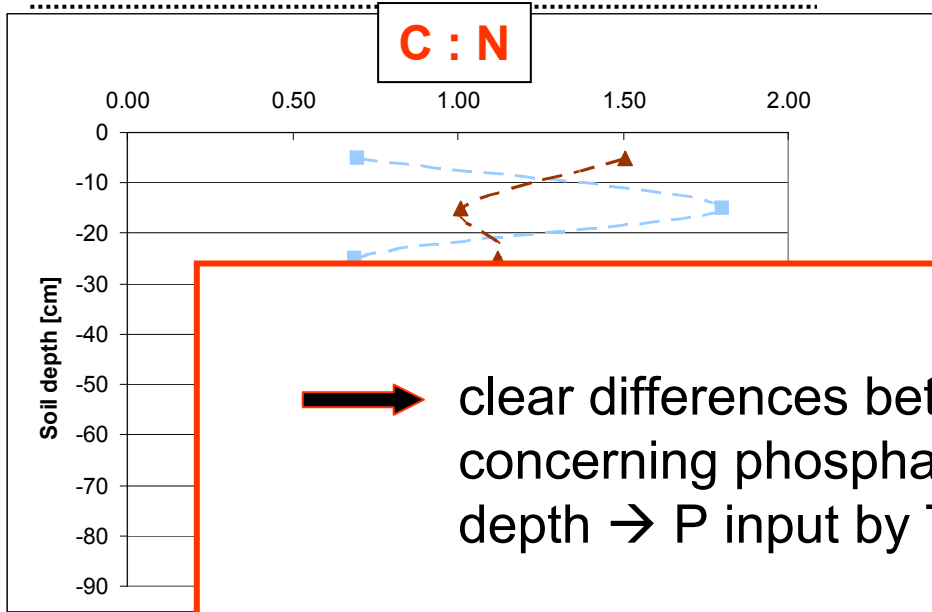
Acco – soil enzyme profiles



Results & Discussion

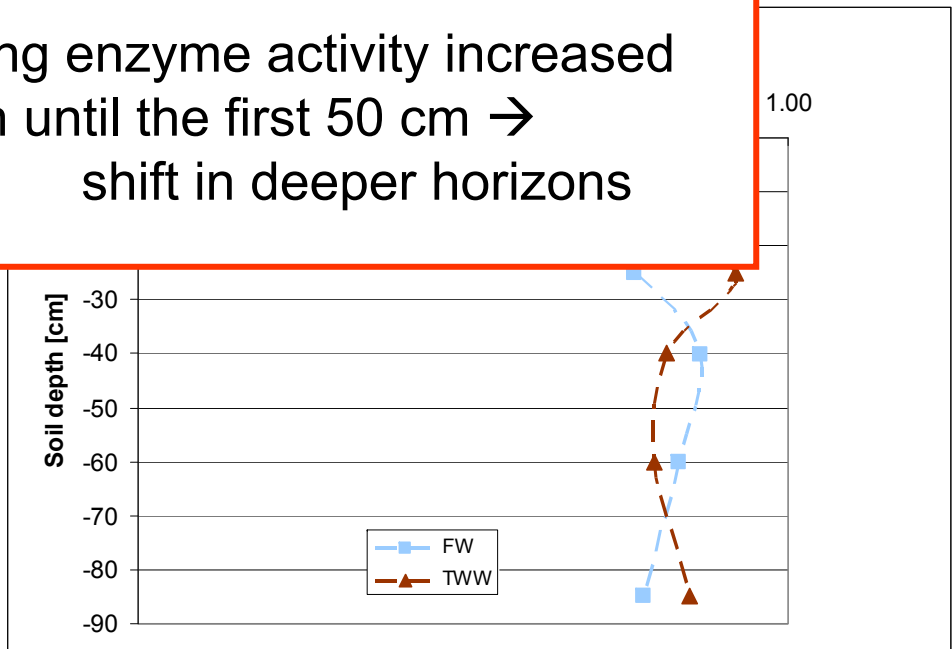
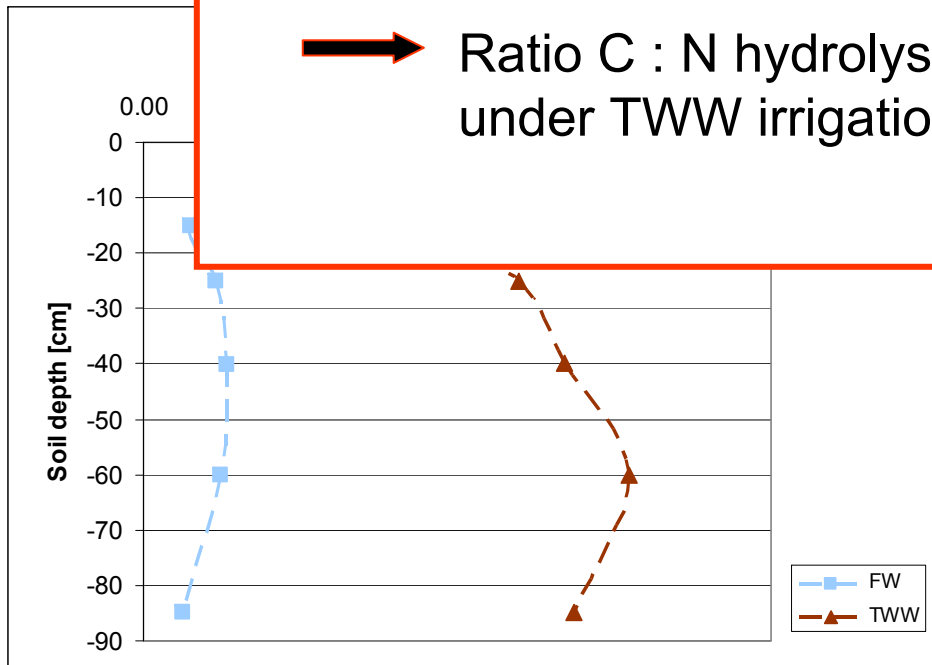
Acco – soil enzyme profiles

Nature Precedings : doi:10.1038/npre.2011.6278.1 : Posted 22 Aug 2011



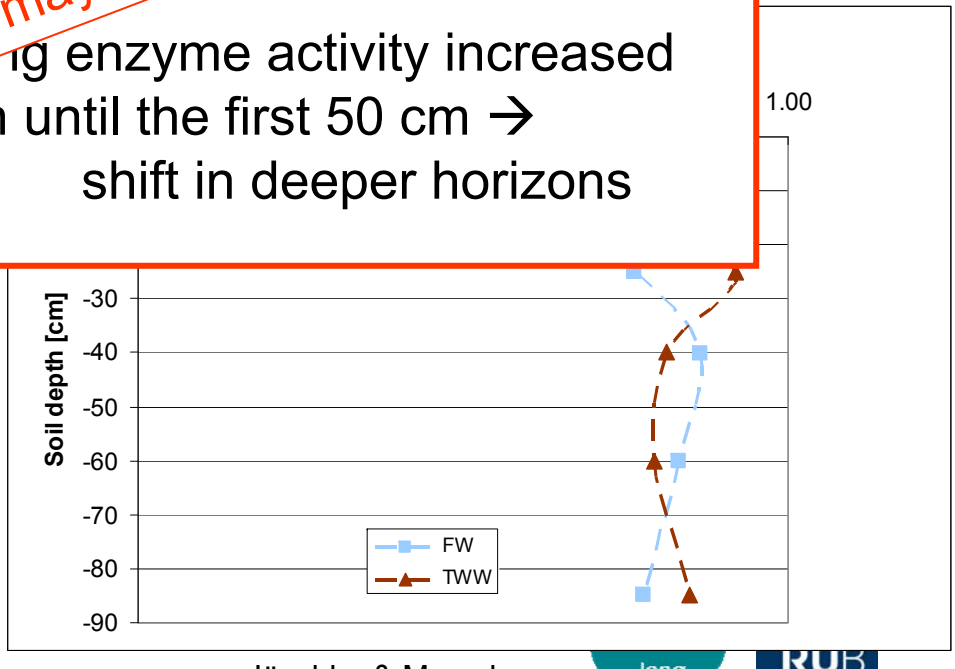
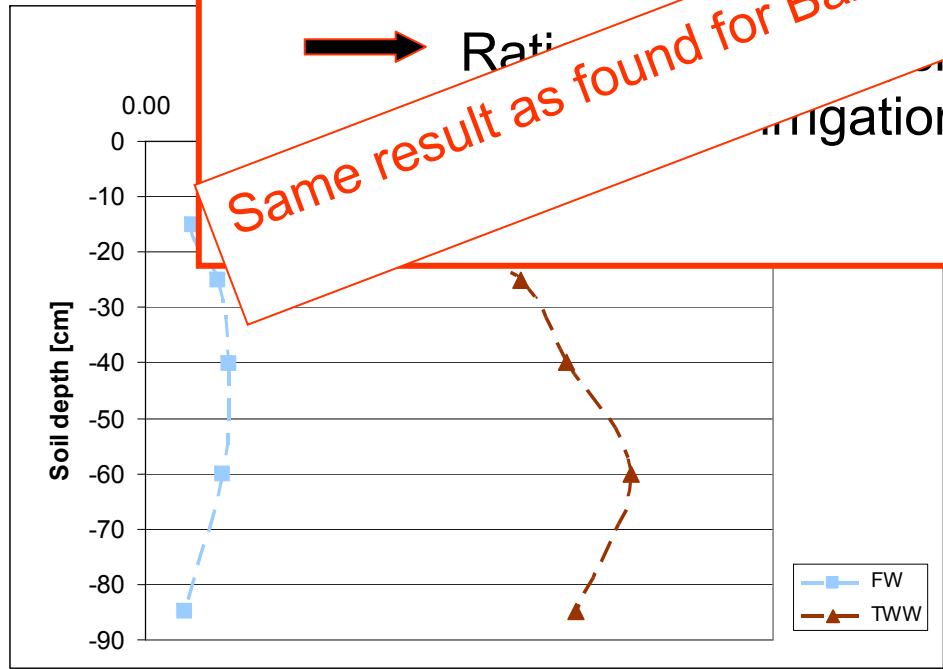
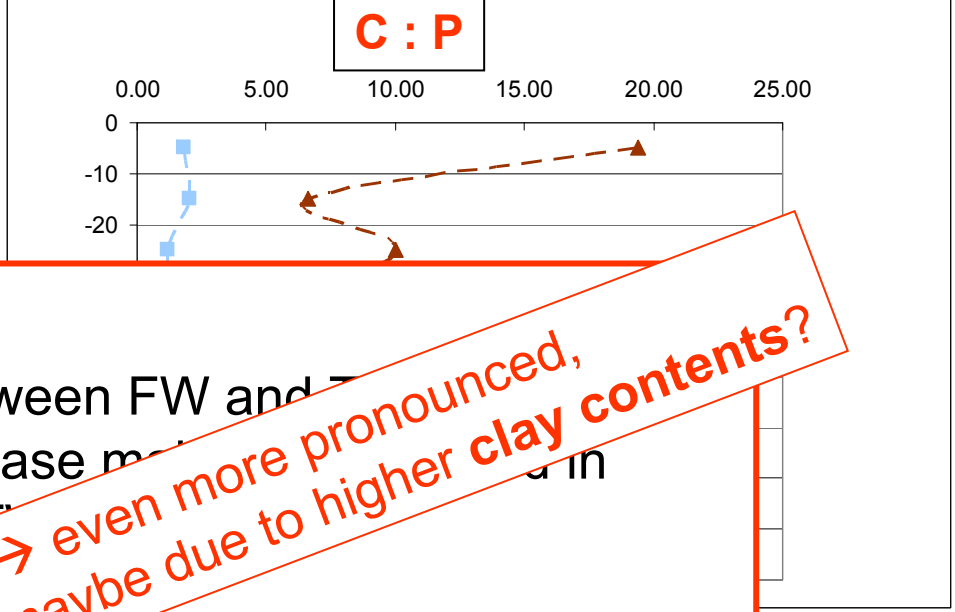
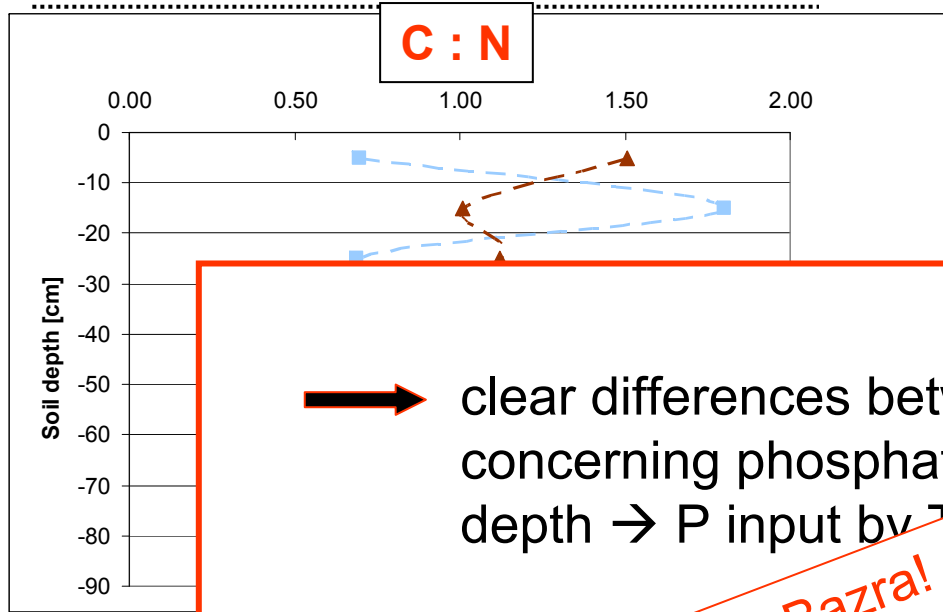
➔ clear differences between FW and TWW concerning phosphatase mainly pronounced in depth → P input by TWW !

➔ Ratio C : N hydrolysing enzyme activity increased under TWW irrigation until the first 50 cm → shift in deeper horizons



Results & Discussion

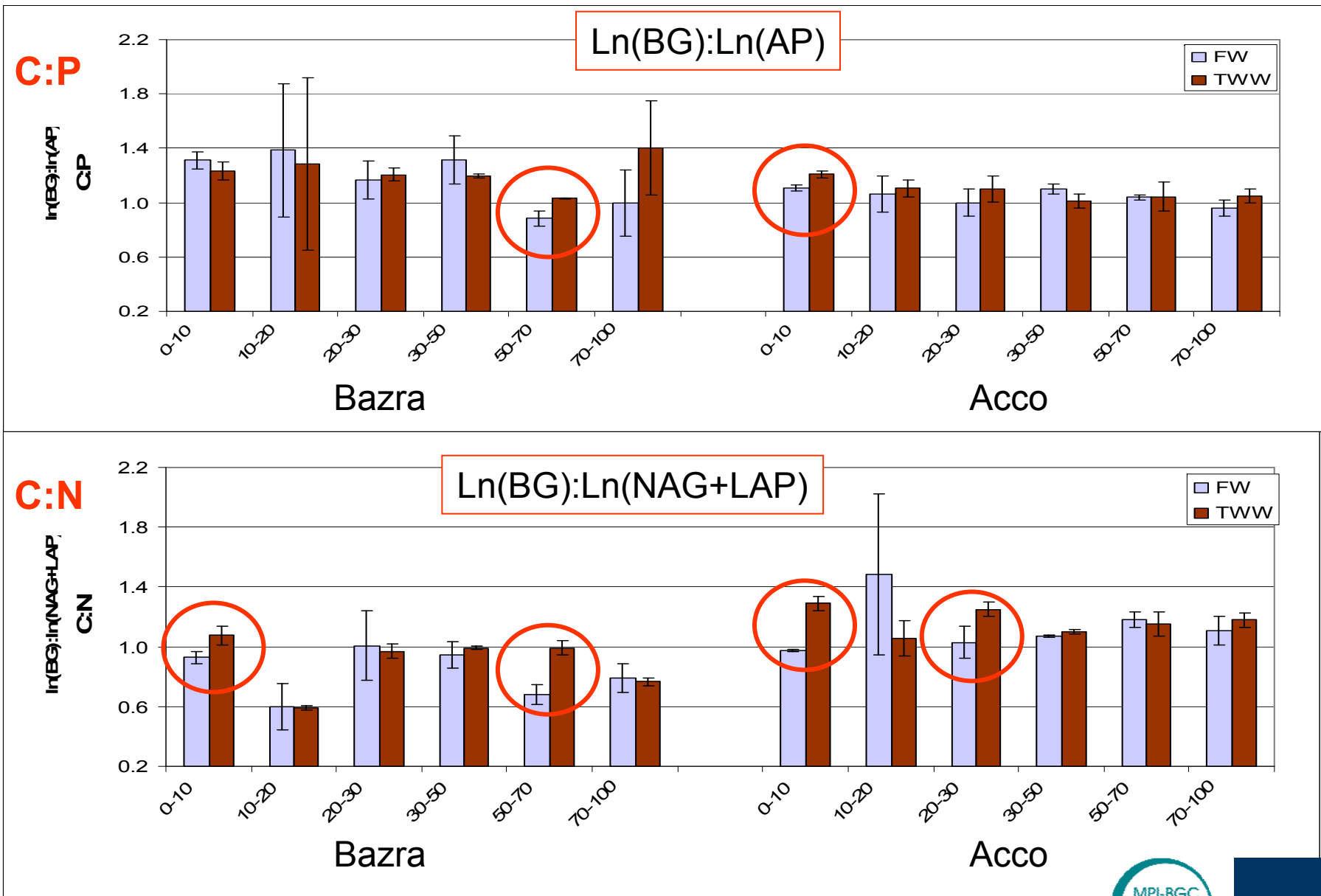
Acco – soil enzyme profiles



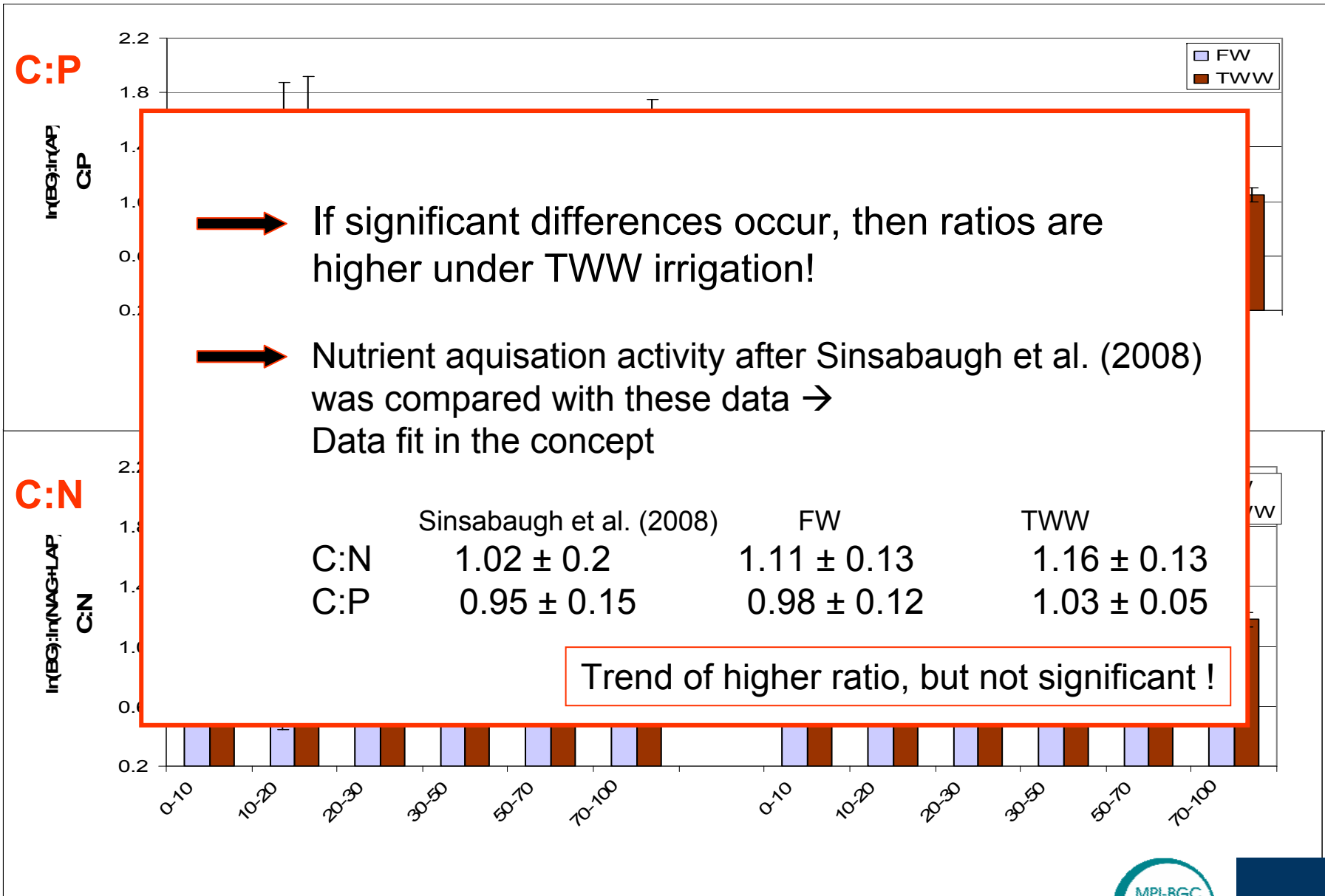
→ clear differences between FW and TWW concerning phosphatase activity in deeper horizons → P input by TWW
 → Ratios of phosphatase activity increased until the first 50 cm → shift in deeper horizons
 Same result as found for Bazra! → even more pronounced, maybe due to higher clay contents?

Results

Nutrient acquisition activity (Sinsabaugh et al. 2008)



(calculated on the basis of g dry soil)



(calculated on the basis of g dry soil)

Summary and Conclusion

Transport of carbon down the soil profiles, which is then used as source for microorganisms

- ↳ Higher total enzyme activity = higher degradation of SOC
- ↳ Continuously triggered priming effects resulted in stronger decrease of SOC → not masked by C input in the subsoil

Decreased phosphatase activity in the subsoil horizons

- ↳ P supply by the TWW is enough, less phosphatase necessary

Clay minerals as possible drivers for enzyme activity (clay bind proteins)

- ↳ Clay-humus-complexes
3-dimensional network
→ active enzymes incorporated (Paul and Mc Laren, 1975)

...before it's open for discussion ...

Thank you for attention

Acknowledgements

- Our cooperation partners: Yona Chen, Yitzhak Hadar (Israel)
Jamal Safi (Gaza Strip)
- My recent workplace, the MPI for Biogeochemistry in Jena, Germany



Picture: drip irrigation system - Elisabeth Jüschke 2004