# EFFECTS OF GRASSED BUFFER STRIP MANAGEMENT ON POTENTIAL DENITRIFICATION IN A BELGIAN AGRICULTURAL WATERSHED

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## ABSTRACT

Riparian buffer strips are managed for the enhancement of water quality through control of non point source pollution. Denitrification in riparian buffer strips is thought to be the major process -with nitrate uptake by plant growth- that reduces nitrate input in surface water. We investigated the Denitrifier Enzyme Activity (DEA) to test how the buffer strip management modifies the denitrification process. The experimental site is composed of a crop field and a 11 m wide grassed buffer strip at the border between the crop field and a tributary to the Attert river, South-East Belgium. Soil samples from the crop field and the buffer strip have been submitted to different imposed conditions combining glucose, nitrate and water saturation to investigate how antecedent water regime, nitrate and carbon content affect denitrification. The work included DEA measurements on undisturbed soil cores freshly sampled. Experiments on undisturbed soil cores identify the buffer strip as more effective in denitrification ($p < 0.001$) than the cropped field. However, experiments on samples conditioned under imposed carbon and/or nitrate contents emphasised the importance of micro-topography.

***KEYWORDS: denitrification, denitirifier enzyme activity, grassed buffer strip, $N_2O$ emission rates, undisturbed soil cores***

## INTRODUCTION

This experimental study takes place in a context of agricultural best management practices linked to the European Nitrate Directive 91/676/EEC. In order to limit the losses linked to agricultural activities, one type of actions promoted by the Nitrates Directive concerns buffer effect of non-fertilised grass strips and hedges along watercourses and ditches.

Some regional program actions for environmental land management have been developed to protect water from diffuse pollution. Consequently, more and more grassed buffer strips have been hence installed between crop fields and watercourses. Most studies have been looking at the global effectiveness of grassed buffer strips in catching excess nitrate. Denitrification in riparian buffer strips is thought to be the major process -with nitrate uptake by plant growth- that reduces nitrate input in surface water.

Quantifying denitrification has a double interest : on one hand there is a need for a better estimation of the impact of grassed buffer strip internal processes on surface water protection, on the second hand, the contribution of agricultural emission of $N_2O$ – gaseous product of the denitrification reaction – to the global warming has to be precised.

This paper presents original results from an experimental study on a buffer strip. The global research project including this study aims at a better management of the diffuse pollution by improving the selection of the locations to be converted in buffer strips.

We compared Denitrifier Enzyme Activity (DEA) of disturbed soil samples from the crop field and the buffer strip. We exposed soil samples to different conditioning treatments affecting water content, C content, and nitrate content. DEA measurements of undisturbed and unconditioned soil samples from the same field plots are also presented.

## MATERIALS AND METHODS

### *Experimental site description*

The research was carried in a 7100 ha agricultural watershed. The experimental site is located along a small tributary of the Attert river, 30 km north-west of the city of Arlon, south Belgium. The mean annual rainfall of this area is 1031 mm.

The studied system is made of a crop field with a maximum slope of 1.2%.

Soil is a sandy silt to heavy sandy silt – cf. table 1 – and is cropped with a rotation maize/ wheat/oats. N fertilisation follows the local agricultural practices : 30 $m^3\ ha^{-1}$ of liquid manure 5% dry matter, 11.3 T $ha^{-1}$ manure and 750 kg $ha^{-1}$ 15-15-15 fertiliser. The grassed buffer strip located between the crop field and the river is 4 years old and 11m wide. It is mowed once a year and not fertilised at all.

**Table 1 : Characteristics of soil samples, carbon content, texture and pH**

| | | % org C | $CO_3^-$ | % Clay | % Silt | % Sand | pH |
|---|---|---|---|---|---|---|---|
| crop field - upper | (plots 1-6) | 1.09 | 1.70 | 10.35 | 50.72 | 38.93 | 6.40 |
| crop field - lower | (plots 7-9) | 0.92 | 1.73 | 8.97 | 55.13 | 35.90 | 6.62 |
| grass buffer strip | (plots 11-12) | 2.39 | 1.90 | 7.05 | 51.90 | 41.05 | 6.46 |

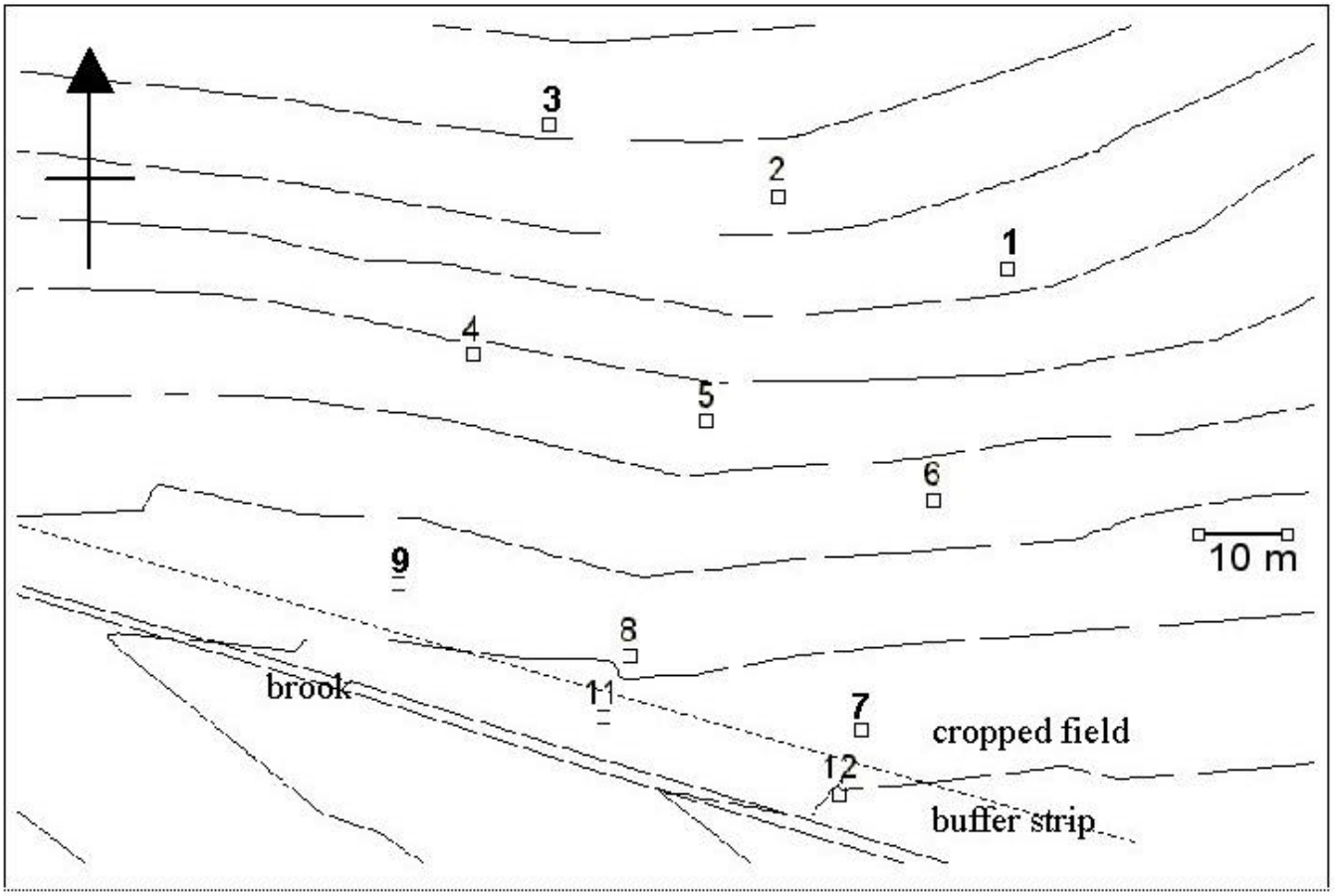


*Figure 1 Location of the plots*

Our experimental grid is 25 square meter wide, as presented in figure 1. So there are 9 plots within the crop field, 25 m from each others and 2 plots in the buffer zone, 15 m far from the first crop plots. Nitrate content is determined weekly by the collection of soil water through porous cup samplers. River content in nitrate is analysed every week.

***Soil sampling and lab experiments***

Collection of soil sample : the top layer (0-5 cm) is sampled in triplicate in February and October 2002. The sampling was realised with a soil auger, with two different types of soil samples: in one case structure was destroyed by rotation of the corer, in the other, small cylinders of 100 $cm^3$ were collected without any effect of rotation. Initial water content is determined.

First experiment:

This experiment is based on the collection of undisturbed soil samples from the top layer (0-5 cm). We collected triplicate soil cores from the 11 field plots. An immediate incubation occurs according to the Denitrifier Enzyme Activity (DEA) measurement method. DEA measurements are made according to the SSSA Methods of Soil Analysis. Slurries of the samples are prepared in a silicone capped glass bottle which allows gas tight syringe sampling (500 µl) of headspace. 30ml solution (1mM chloramphenicol, 1mM glucose and 1mM $KNO_3$) is added till saturation to forbid de novo synthesis of enzyme, and to make sure that the denitrification reaction will not lack of carbon or nitrate .

Then, anaerobiosis conditions are imposed by a 5 minutes $N_2$ flush. According the acetylene inhibition technique, 10% volume of acetylene is added to block the transformation of $N_2O$ to $N_2$.

Analysis of $N_2O$ is performed on a GC equipped with a electron capture detector (ECD). Each bottle is analysed every 15 minutes, during one hour and a half. The production rate of $N_2O$ is obtained upon the regression slope of the evolution of $N_2O$ concentration vs. time (cf. fig.2).

Second experiment:

This experiment is partly based on the investigation methods of denitrification dynamics presented by Dendooven et al. in 1999. Soils samples are sieved to pass 6.5 mm, then homogenised and 3 sub-samples are weighted. Each sub-sample is then conditioned aerobically for one month at 25°C before measuring its Denitrifier Enzyme Activity (DEA).

Conditioning conditions are as follow :

- 150 ml of distilled water (wat)
- 150 ml of a solution of 10mM $KNO_3$ (kno3)
- 150 ml of a solution of 10mM $KNO_3$ and 4mM glucose (glu)

The DEA solution (1mM chloramphenicol, 1mM glucose and 1mM $KNO_3$) is added and the sample incubated in screw-cap glass bottles. The caps contained gas-tight septa through which 500 µl gas samples were withdrawn with a gas-tight

syringe for $N_2O$ analysis. The headspace $N_2O$ concentration is recorded during 2 hours in the same way as in experiment one.

***Statistical analysis :***

As the results are distributed lognormally, we employed Parkin's LSTAT soft to characterise the samples mean, variance and standard deviation.

**Results**

*First experiment:*

Global trends for $N_2O$ production rates in mg $N_2O$-N $kg^{-1}$ $d^{-1}$ are presented in figure 2, showing time evolution of $N_2O$ concentration in the headspace of the incubation bottles. In addition to the expected linear aspect of the relation, we can notice that the plots 11 and 12, from the buffer zone, are distinctly above the others, plot 9 coming just afterwards.

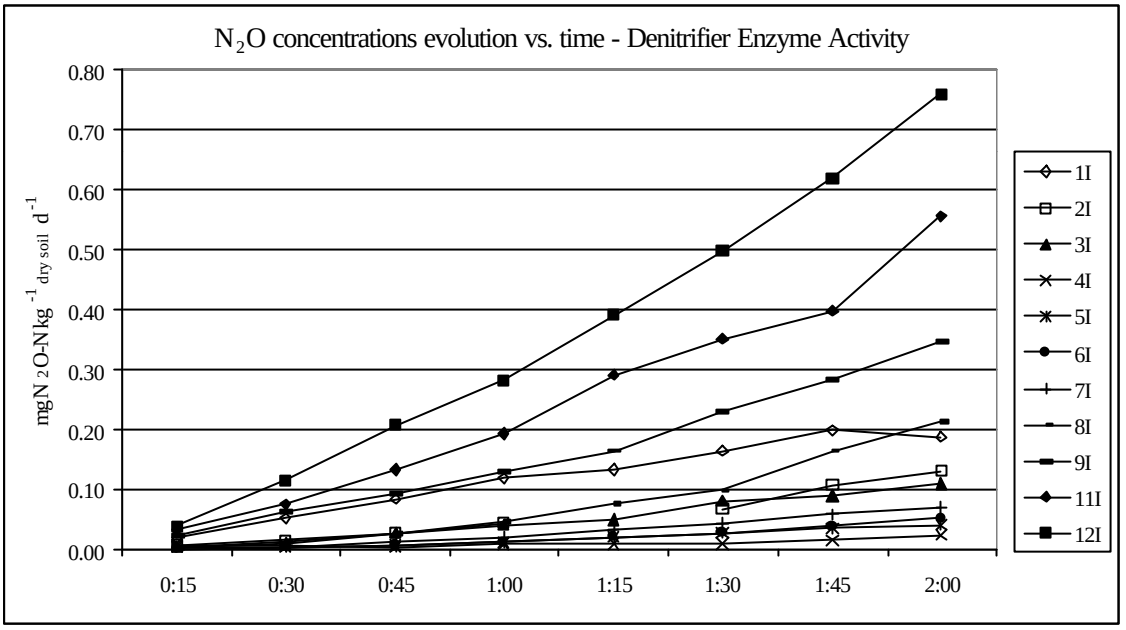


*Fig.2 : DEA measurements on undisturbed soil samples from 11 plots (plots 1 to 9 from the crop field and plots 11 and 12 from the buffer strip).*

$N_2O$ production rates characteristics for undisturbed soil samples are presented in table 2. The five highest rates come from grass buffer strip samples (plots 11 and 12) and run from 11.05 to 6.88 mg $N_2O$-N $kg^{-1}$ $d^{-1}$. The five lowest production rates were measured on samples from the upper part of the crop field (plots 3, 4 and 5).

***Table 2 : Mean production rate in mg $N_2O$-N $kg^{-1}$ $d^{-1}$ for the upper and lower parts of the crop field and for the buffer zone. Mean and variance according to UMVUE/LAND estimators, LSTATS, Parkin.***

| | Upper crop field | Lower crop field | Grass buffer strip |
|---|---|---|---|
| Mean production rate | 0.86 | 2.40 | 8.38 |
| Variance | 0.65 | 3.83 | 22.15 |

High variance values due to variability of the denitrification process is better presented in table 3. Triplicate samples of the buffer strip plots (11 and 12) and their respective production rate have similar mean for highly dispersed results.

***Table 3 : $N_2O$ production rate detail for buffer zone plots. Rep. is for replicate. Mean and variance according to UMVUE/LAND estimators, LSTATS, Parkin.***

| | Rep. I | Rep.II | Rep.III | Mean | Variance |
|---|---|---|---|---|---|
| Plot 11 | 6.8802 | 10.0800 | 2.4619 | 6.57 | 20.29 |
| Plot 12 | 9.7893 | 8.6886 | 11.0466 | 9.84 | 1.39 |

*Second experiment:*

GLU-incubated samples produced the highest $N_2O$ production rates. The mean production rate for the crop field GLU-samples is more than fifteen times greater than that of the undisturbed soil cores (25.03 mg $N_2O$-N $kg^{-1}$ $d^{-1}$). The mean production rate for the buffer strip GLU-samples is 34.91 mg $N_2O$-N $kg^{-1}$ $d^{-1}$. Figure 2 presents triplicate results for the whole group of samples : undisturbed, glu-, kno3- and wat-incubated.

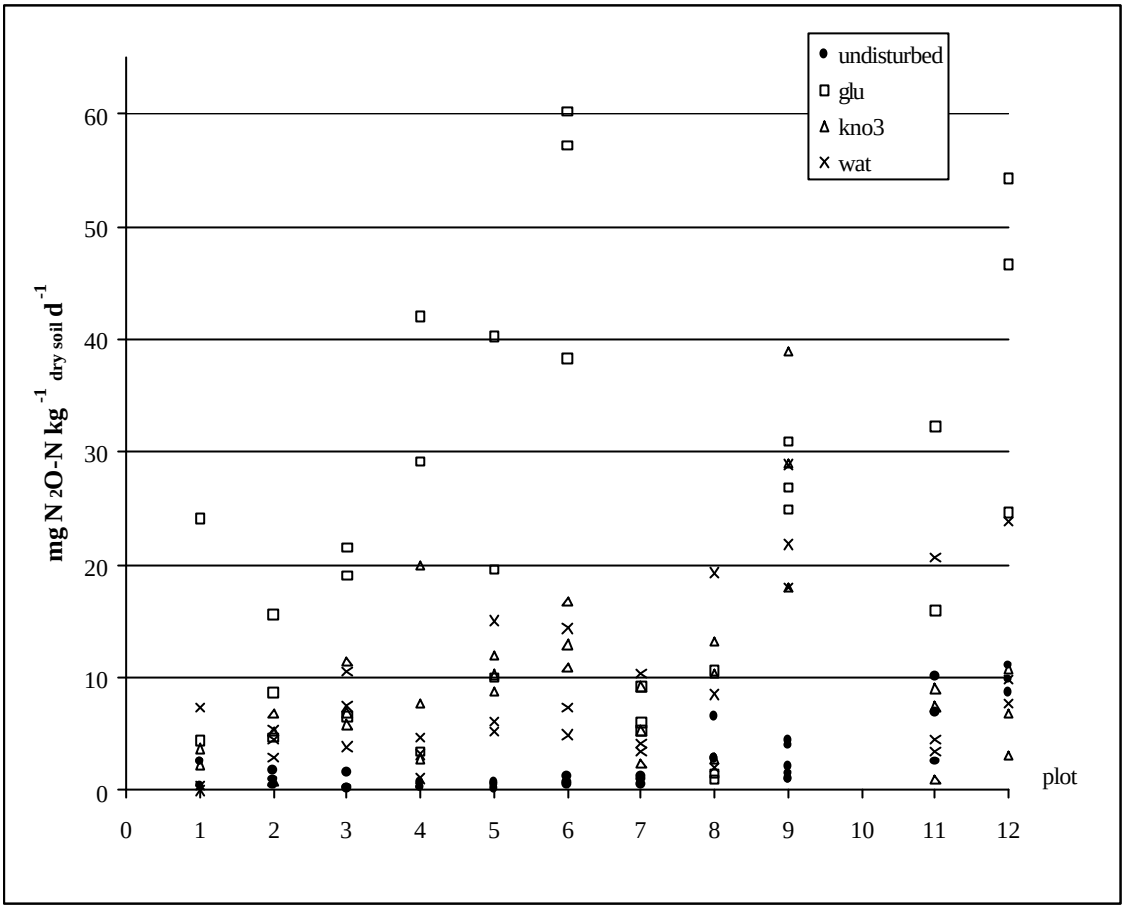


*Fig.3 : $N_2O$ production rates (DEA) for both experiments. Triplicate results are presented for each of the treatments (undisturbed, glu, kno3 and wat) and for each plot (from 1 to 9 and 11 to 12).*

Semivariogram helps to analyse the results which show typical lognormal distribution. It means peer to peer plotting variance versus distance. The variance of the samples conditioned with glucose and nitrate (glu treatment) is twice the one of the nitrate-only added samples (kno3). In the same way, the variance of soil samples oversaturated with water (wat ; no nitrate or glucose but what was initially contained in the soil) are twice lower than the nitrate-only conditioned samples. No obvious distance effect appears in these four diagrams.

## DISCUSSION

Some questions about the high variability between replicates (cf. table 3) can't find any answer in measuring the oxydable carbon content –whatever it was a key parameter as shown by the second experiment (cf. fig.3). Since replicates sample (plots 11 and 12) were collected so close to each others -a few centimeters- we have to conclude that even if the buffer strip shows higher rates of denitrification (mean production rates are significantly different at $p < 0.001$), the distribution of the active micro-sites seems to be the main factor that explain the variability of the results. However, topographic position of the plots (upper or lower crop field, buffer strip) is related to the denitrifier enzyme activity when samples are kept as undisturbed as possible : structure-preservative sampling, immediate incubation (no storage).

The semivariograms do not show dependence between the variance of measurements and the position of the samples in space. However, semivariogram "KNO3" distinguishes a small group from point-variances definitely far away from the majority of the points (semivariances roughly speaking from 140 to 401 against 0 to 75). These outsiders correspond to the comparison between sample 9 and all the others. The same report can be drawn up for semivariogram "WAT" where 7 out of the 11 points which are dissociated from the others (variances roughly speaking between 90 and 210) belong to pairs of points including plot 9. Moreover this group of points presents positive trend of the variance according to the distance between points.

The different $N_2O$ production rates (figure 3) are mainly due to the different treatments. The glu treatment causes a clear increase in the production rate for all plots but plot 9. Mean production rates of plot 9 is significantly ($p < 0.0025$) higher than the rates of the other plots for wat and kno3 treatments whereas it is not significantly different for the glu treatment. It means that denitrification potential is high for each sample once put in the presence of organic carbon and nitrate.

The plot 9 is surprisingly located in the crop field although we could have expected such a behaviour for buffer strip samples. However plot 9 is close to the buffer strip, at the bottom of the cropped area. Moreover sampling followed one 5 days period of rain. We noted that square 9 was the only one gorged with water. A later visit in the field confirmed that the local micro-topography induced a concentration of the surface runoff precisely to plot 9.

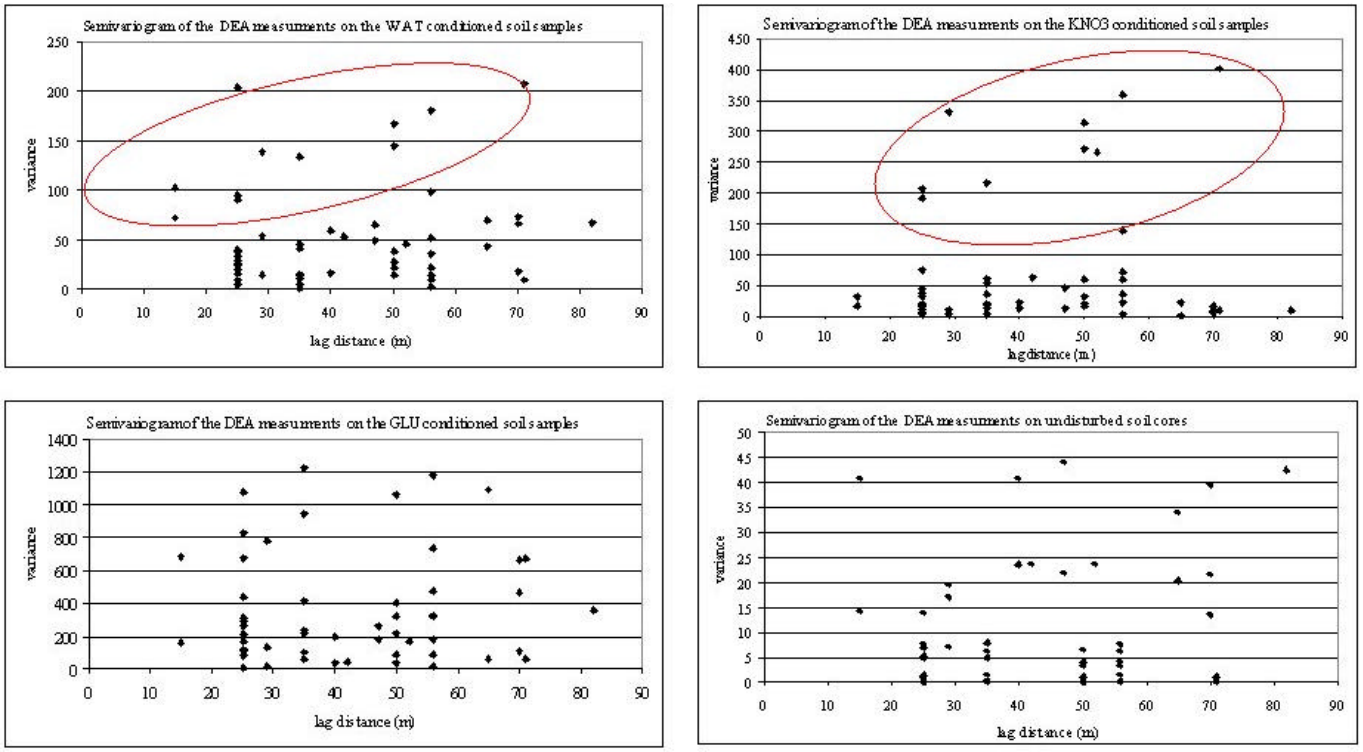


*Fig.4 : semivariograms of $N_2O$ production rates (DEA) for both experiment. Lag distances are expressed in meters and are related to the actual distance between plots. Each point refers to peer to peer variance between plots. The line that circles some points in the wat and the kno3 diagrams points out the plot 9 comparisons to the others.*

## CONCLUSION

Experiments on undisturbed soil cores identify the buffer strip as more effective in denitrification ($p < 0.001$) than the cropped field. However, the experiments of samples conditioning under imposed conditions of carbon and/or nitrate contents emphasised the importance of micro-topography. Surface runoff indeed seems to have a greater influence on DEA than the incubation treatments.

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