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The self-purifying capacity, measured as the biodegradation of acetate, is an important ecosystem-service in the upper groundwater zone in the Netherlands

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In memoriam Peter Doelman

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Abstract

The self-purifying capacity, measured as the biodegradation of acetate, is an important ecosystem-service in the upper groundwater zone in the Netherlands

The upper layer of the Dutch groundwater has the capacity to remove its own pollution. Therefore this groundwater can be used safely as drinking water and it does not threat soil or surface water ecosystems. The RIVM research which measured the self purifying capacity on 128 locations in the Netherlands, shows that this capacity is a reliable ecosystem service. It is less subjected to disturbances than the self purifying capacity of the soil, which can be inhibited by drought and low temperatures. Certain pollutions however can decrease the self purifying capacity. This is the case for the pollutions which can inhibit the microorganisms that perform the self purifying capacity.
A new experimental method has been developed during the RIVM research, which can measure and map the self purifying capacity of the subsoil. This method which measures the conversion rates of acetic acid, is easier and quicker than the previously used methods. In order to compare the different locations, these rates were plotted against the depth, soil type, conductivity and nitrate content. Especially the upper groundwater zone of the peat soils in the west of the Netherlands exhibited a large self purifying capacity. Often this is even larger than that of surface soil. These peat soils are chalked meadows with a high groundwater level and a low nitrate content. The self purifying capacity often is a factor 30 lower in the sandy soils in the eastern parts of the country.

Keywords:
groundwater, biodegradation, the Netherlands, acetate, ecosystem services, self purifying capacity

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Rapport in het kort

Experimentele meetmethode voor zelfreinigend vermogen in bovenste grondwaterzone in Nederland

De bovenste laag van het grondwater in Nederland is in staat om zelf verontreinigingen op te ruimen. Hierdoor kan het grondwater veilig voor drinkwaterwinning worden gebruikt en bedreigt het de ecosystemen van de bodem en het water niet.
Het RIVM onderzoek waarbij op 128 locaties in Nederland het zelfreinigend vermogen is gemeten laat zien dat dit vermogen een betrouwbare ecosysteemdienst is. Het staat minder bloot aan verstoringen dan het zelfreinigend vermogen in de bodem, dat door droogte en lage temperaturen kan worden afgeremd. Wel zetten bepaalde verontreinigingen het zelfreinigend vermogen onder druk. Het gaat dan om verontreinigingen waarvoor de micro-organismen die verantwoordelijk zijn voor het zelfreinigend vermogen gevoelig zijn.
Tijdens het RIVM onderzoek is een nieuwe experimentele methode ontwikkeld, waarmee het zelfreinigend vermogen in de ondergrond kan worden gemeten en in kaart gebracht. Deze methode die de omzettingssnelheden van azijnzuur meet, is eenvoudiger en sneller dan tot nu toe gebruikte technieken. Om het zelfreinigend vermogen van de onderzochte locaties te kunnen vergelijken zijn deze snelheden vervolgens afgezet tegen de diepte, de grondsoort, de geleidbaarheid en het nitraatgehalte. Vooral de bovenste grondwaterzone van de veengronden in het westen van Nederland vertonen een groot zelfreinigend vermogen. Vaak is dit nog groter dan dat van de bovengrond. Deze veengronden zijn bekalkte weilanden met een hoge grondwaterstand en een laag nitraatgehalte. In de zandgronden in het oosten van het land is het zelfreinigend vermogen vaak een factor 30 lager.

Trefwoorden:
grondwater, biodegradatie, Nederland, acetaat, ecosysteemdiensten, zelfreinigend vermogen

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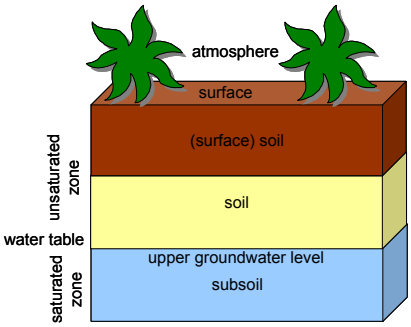
Summary

We have developed a new experimental method to measure the self purifying capacity of subsoils and soils. This method is easier and quicker than the techniques that were previously used. Only a minute amount of tritium labeled acetate and water are added to subsoil material. After an incubation time, the subsoil material (also containing now radioactive bacteria) is separated from the water by centrifugation. In the next step the formed tritium labeled water is separated from the remaining acetate by evaporation.
The method was used to measure the acetate mineralization rate in the upper groundwater zone of 128 locations in the Netherlands. The data were plotted in a soil map of the Netherlands and were also compared with the depth, dry weight, electric conductivity, pH and nitrate concentration. The peat areas consisted of chalked meadows with a high groundwater level whereas the sand areas often showed deeper groundwater and lower pH values. The upper groundwater zone of the peat areas showed a mineralization rate which was even higher than that of surface soils. In contrast, the mineralization rate of the upper groundwater zone of sandy soils showed on average a factor 30 lower rate.

1. Introduction

1.1. European legislation demands groundwater protection

The European water framework directive (EC, 2000) and the groundwater directive (EC, 2006) state that the European Member States must take measures to limit or prevent the input of pollutants in groundwater. The European guidance number 17 prevent and limiting direct and indirect inputs (EC, 2007) describes points of compliance. At these points it must be checked whether the groundwater meets quality criteria. The first point of compliance lies directly under the pollution source at the upper layer of the groundwater saturated subsoil. We will use the word subsoil for the solid materials under the water table together with the groundwater. For dangerous substances the environmental quality standard has to be met at this point of compliance (EC, 2007). For non-dangerous substances the quality standard does not need to be met at this point but must be met down streams of the groundwater. Biodegradation, sorption and dilution can play a role in this process.



1.2 The self purifying capacity is a vital ecosystem service

The self purifying capacity of the upper groundwater level of the subsoil plays an important role because of the favorable conditions for biodegradation in this layer. In surface soil however, extreme temperatures and drought can often limit biodegradation. Moreover, during thunderstorms massive amounts of water can suddenly pour down to the groundwater. The same can happen during snowmelt in spring. Therefore the self purifying capacity of the upper groundwater zone is a vital ecosystem service. This self purifying capacity is not only important for the removal of pollutants (Van Beelen, 1990), but also plays an important role in the removal of pathogens from drinking water (Schijven et al., 2006). Natural compounds which are not degraded can end up in drinking water tubes and facilitate the growth of pathogens (van der kooij and Hijnen, 1985). These natural compounds can also stimulate the formation of chlorinated hydrocarbons when the drinking water is treated with chlorine (Medema and Havelaar, 1994). The self purifying capacity is vulnerable to soil pollution (Van Beelen et al., 1990, Van Beelen et al., 1991) and improper groundwater management. Therefore a research project was commissioned by the Dutch Ministry of Housing, Spatial Planning and the Environment to study the self purifying capacity of subsoils in the Netherlands.

1.3. Requirements for a test measuring the self purifying capacity

The project had to develop a measuring method for the self purifying capacity. This measure should be simple and rapid enough to determine the normal self purifying capacity of the subsoil in the Netherlands. Therefore we searched for a simple and rapid test to evaluate the capacity of the subsoil to degrade organic compounds. The test would need to include both the groundwater and the accompanying subsoil since most bacteria in the groundwater zone are attached to particles and do not float freely in the groundwater (Marxsen, 1981). Our interest is focused on the biodegradation at micrograms/liter concentrations in relatively unpolluted subsoils. At these low concentrations there is no growth of the original microflora during the experiment. This resembles the natural conditions of the microflora in the subsoil where the

substrate concentrations are normally too low for growth. The allowable pesticide concentrations in the European Union are below 0.1 µg/l. The degradation of pesticides in groundwater is however relatively slow and depends strongly on the specific pesticide. Therefore we selected a simple and rapidly degradable compound. We did use the mineralization of radiolabeled ^{14}C acetate into $^{14}CO_2$ previously, to monitor biodegradation in groundwater (Van Beelen et al., 1991). The method of Bååth using tritium labeled compounds seemed more practical since the safety procedures allow the experiments to be performed in a normal laboratory (Aldén Demoling and Bååth, 2008). This method demanded multiple washing steps of a radioactive pellet which were too laborious and difficult for our purposes. We were able to simplify this method considerably using a single centrifugation step to separate the remaining acetate and the formed $^{3}H_2O$ from the subsoil or the soil.

2. Materials and methods

2.1. The new method to measure the activity in subsoils

A stock solution of tritium labeled acetate in ethanol was diluted with non-labeled acetate to a specific activity of 2 GBq/mmol. The ethanol from the stock solution was removed by evaporation. The subsoil samples were taken at the groundwater table with a sand type Edelman auger (Eijkelkamp, the Netherlands). During the sampling the subsoil was not touched or polluted with surface soil. Heating or drying of the sample was avoided. The samples were stored in closed plastic bags at 4 °C for less than three months.
For the experiments 15 g of wet peat subsoil was homogenized in 150 mL water. For the sandy subsoils and equal mix of 150 g wet sand and 150 mL water was used. A household blender was used at maximum speed for 30 seconds for the homogenization. The subsoil was allowed to settle for 10 minutes. 50 mL of the upper suspension was taken from the blender. This suspension was continuously mixed while 1.35 mL subsamples were put in 2 mL Eppendorf tubes. Typically 8 duplicate tubes were used for a time series experiment.
150 µl of the tritium labeled acetate was added to reach a final volume of 1.5 mL. In the samples a final concentration of 1 Bq/µl was used giving 41 µg acetate/liter (0.5 µM). The solid matter concentration in the peat subsoils was generally about 30 g dry weight/liter while in the sandy subsoil concentration it was about 500 g dry weight/liter. The shortest incubation time was 15 minutes and the following times were always a factor 3 longer. Therefore the seventh incubation time was 182.25 hours. For the incubation times longer than four hours the Eppendorf tubes were stirred on a roller bench to avoid settling of the solid matter. The room temperature incubation was stopped in an Eppendorf centrifuge at 14,000 RPM (20 000 g) for 10 minutes at 5 °C. The start of the run was taken as the stopping time. An amount of 300 µl from the supernatant was put in a counting vessel and 10 mL Ultima Gold LLT was added. Another amount of 300 µl was put in a counting vessel with 400 µl ethanol containing 10 mM NaOH. The ethanol was added to stop microbial growth while the sodium hydroxide lowered the pH to prevent acetic acid evaporation. These vessels were dried overnight in a fume box to remove ^3H_2O. After drying also 10 mL Ultima Gold LLT was added in these ^{3}H-acetate vessels. The tritium radioactivity was counted for 1.5 minutes per vessel in a Tricarb scintillation detector from Packard.

2.2. Data analysis

Figure 1 shows a typical example of the half-life determination for a single subsoil. The triangles represent the total counts in the supernatant. That is the remaining labeled acetate and the formed labeled water. Even at the shortest possible incubation times the total count does not amount to the theoretical maximum of 300 Bq. Apparently there is about 50 Bq (17%) of the labeled acetate bound to the pellet. At longer incubation times even more labeled acetate (37%) is bound to the pellet. This is probably due to the uptake and incorporation of labeled acetate into the subsoil bacteria. The circles show the remaining labeled acetate. Even at the shortest incubation times the circles are below the triangles. This indicates that the stopping procedure using the centrifuge was not rapid enough to prevent an initial small conversion of acetate into labeled water. The data are however well-suited to derive the half-life of acetate. The lower curve was fitted through the circles using nonlinear least squares with the following formula:

Y=300*exp(-ln(2)*X / hl))
Y = the expected counts in becquerel
X the time in minutes
hl = the half-life of the mineralization

For this experiment with subsoil 10 it was 90 minutes with a standard error of 13 minutes. The data were fitted using nonlinear regression (Bates and Chambers, 1992) with the statistical program R. The upper curve was drawn according to:

Y=M+(Max-M)*exp(-ln(2)*X / hl)

Y = the expected counts in becquerel
X the time in minutes
hl = the half-life in minutes of the mineralization taken from the lower curve
Max = the maximal amount of total becquerels in the whole data set
M= the minimal amount of becquerels reached after a long incubation time

For this curve only the value of M was estimated. In fact, the upper curve with the triangles showing the total becquerels were not used for the half-life estimation. They were only used as a check for the total radioactivity. Figure 1 was automatically drawn in R (script available by the author).

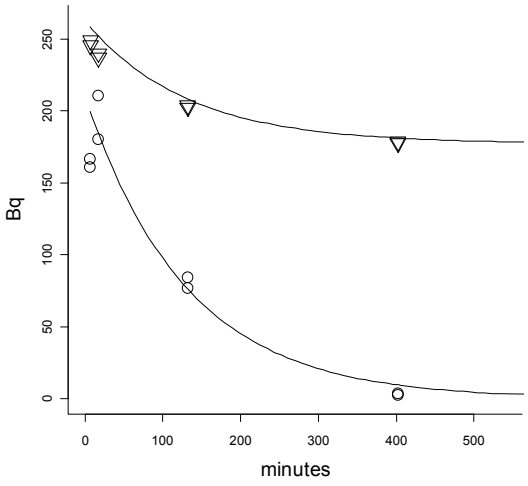


Figure 1: An example of the measurement of the half-life of acetate due to mineralization. The triangles around the upper line show the total amount of radioactivity in the supernatants while the circles around the lower line show the radioactivity of the labeled acetate after evaporation of the ^3H_2O. In the beginning of the experiment 250 Bq ^3H acetate was present which was converted to ^3H_2O after 400 minutes.

Since 128 different subsoils were sampled it is not feasible show all the graphs. Therefore the results of the data fitting are presented in Table 1 at the end of this report. Maxipercent is the maximal percentage of acetate still present at the shortest incubation time. The half-lives with a corresponding maxipercent <10% are unreliable because most of the acetate was already mineralized at the shortest incubation time. The unreliable data were removed from the analysis. For samples before number 55 an amount of 100 gram subsoil/100 mL water was used while for the samples after number 55 only
15 grams subsoil/150 mL water was used. This dilution was performed to slow down the mineralization because otherwise all the acetate was already converted at the shortest possible incubation time. Note that the standard error is generally in proportion with the half-life. For this reason we used logarithmically transformed mineralization rates in the statistical analysis. This gives a more constant standard error over the whole range of mineralization rates.

2.3. Calculation of normalized mineralization rates from half-lives

Equal amounts of fresh subsoil and water were mixed in order to determine the total volume of the wet soil. This volume was needed to calculate the amount of solid matter from the subsoil in 1.5 mL in the Eppendorf vessels. The fresh subsoils did contain water but no air. We assumed that the activity of the bacteria in the slurry was not altered by the immediate precipitation of the sand. Therefore we corrected the acetate

mineralization rate by the bacteria with the total dry weight of the subsoil. When 100 g fresh sandy subsoil was mixed with 100 mL water the final volume the slurry was called “v” mL. The dry weight of 100 g fresh sandy subsoil is “dw” gram. The slurry contains dw / v grams dry weight/mL slurry. Therefore an Eppendorf vessel with 1.5 mL of the above slurry contains 1.5 * dw / v grams of dry weight subsoil solid matter. The rate r of the acetate mineralization can be calculated using:

r = 1000 * ln(2) * v / (hl * dw)

r = rate expressed in mL / (minute * kg)
ln(2) = 0.6931
v= volume 1.5 mL per vessel
dw= grams of dry weight subsoil solid matter per vessel

This formula is used to normalize the rates to the amounts of subsoil solid matter used. For experiment number 10 for example hl = 90 minutes, v = 154 mL, dw = 83 grams and therefore
r = 14 mL/(min*kg).

3. Results and discussion

3.1. The acetate mineralization rate is rapid in peaty subsoils

The mineralization rate of acetate was measured in subsoil samples from the groundwater table at 126 locations in the Netherlands. The rate was corrected for the amount of subsoil solid matter used in the experiment. For sandy subsoils a soil/water ratio of 1 gram fresh weight/1 mL was used while for peat subsoils and surface soils a ratio of 1 gram soil / 10 mL water was necessary. In the latter soils the acetate mineralization was very rapid and the extra dilution slowed down the process so we were able to measure it after the first 10 minutes centrifugation step.

Figure 2 shows the sampling locations on the physical geographical map of the Netherlands. The locations are in the middle of the drawn circles. The size of the circles correspond with the acetate mineralization rate ranging from 1.5 upto 2412 mL * min^{-1} * kg^{-1}. On a logarithmic scale this ranges from 0.2 up to 3.4. The sandy subsoils in the North Eastern part of the Netherlands show a lower mineralization rate than the peat subsoils in the lower laying Western parts. Many samples were taken close together within one farm. The map shows that most farms have either big circles or small circles. This indicates that the method is reproducible and that close by samples are similar. In Figure 2 the lower mineralization rates are presented as dots and are therefore not very clearly visible. These low mineralization rates are derived from samples with a large half-life. These were generally very white sands.

Figure 3 is shown in addition to Figure 2 to put emphasis on the subsoils which have a low mineralization rate and therefore a larger half-life. These subsoils are probably more vulnerable to pollution because they have very limited capacity to degrade even the simplest of organic compounds. The small circles in Figure 2 are now presented as large circles in figure 3 and vice versa.

Table 2 (presented at the end of the report) shows a summary of the data. Samples from the same farm have the same letter in the code. The samples are presented in the measured order, except number 73 through 80 which were surface soils from a laboratory experiment performed in the University of Utrecht. The samples 82, 83 and 126 were also presented at the end of the table because the conductivity was not measured.

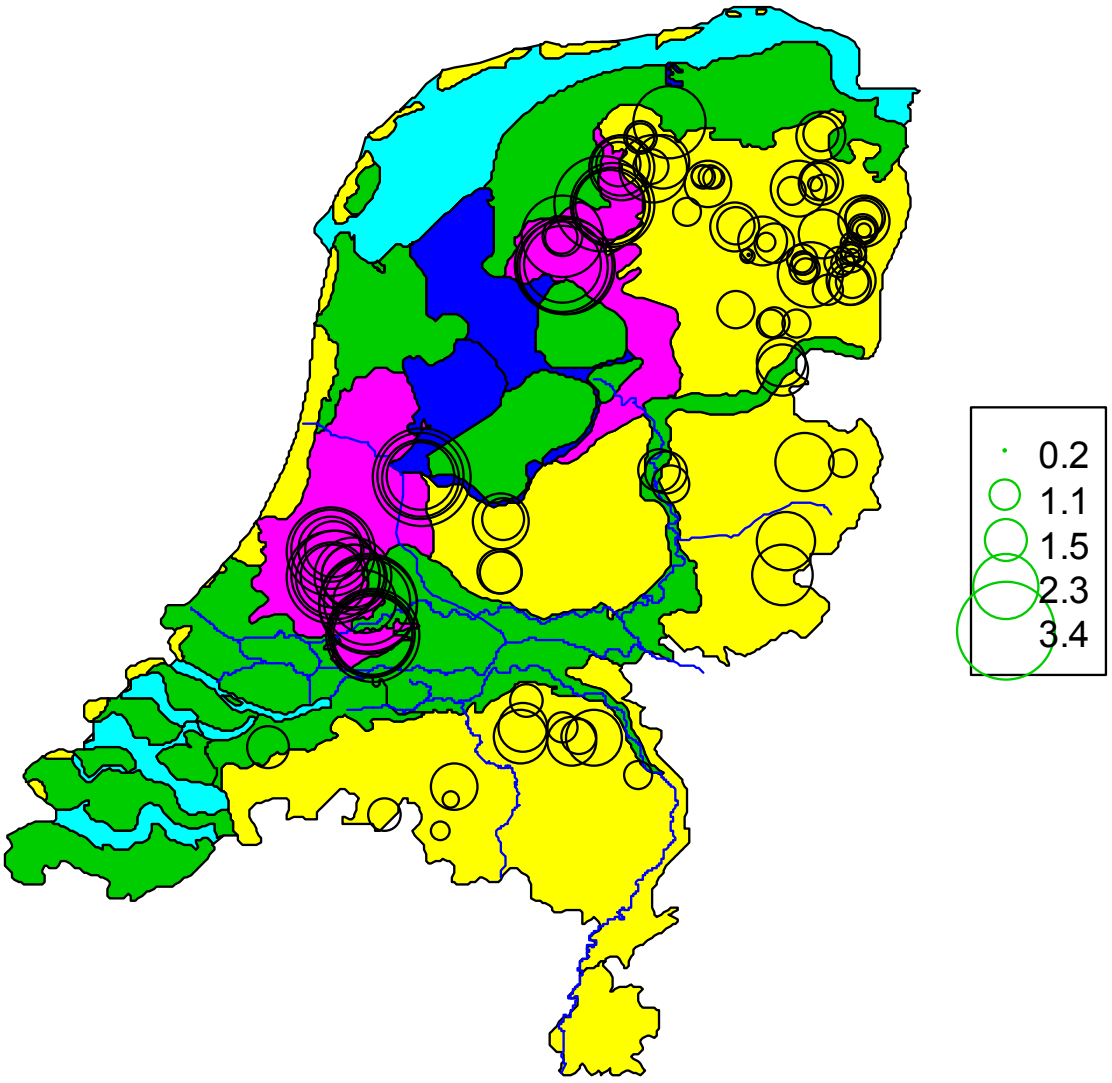


Figure 2: The acetate mineralization rate at the groundwater table in the Netherlands. The circles indicate the sampling locations. Larger circles indicate a faster logarithmically transformed mineralization rate. The yellow areas are the higher sandy soils while the pink areas are peat soils located in the lower parts of the Netherlands. The clay soils are green, the fresh water blue and the salt water light blue.

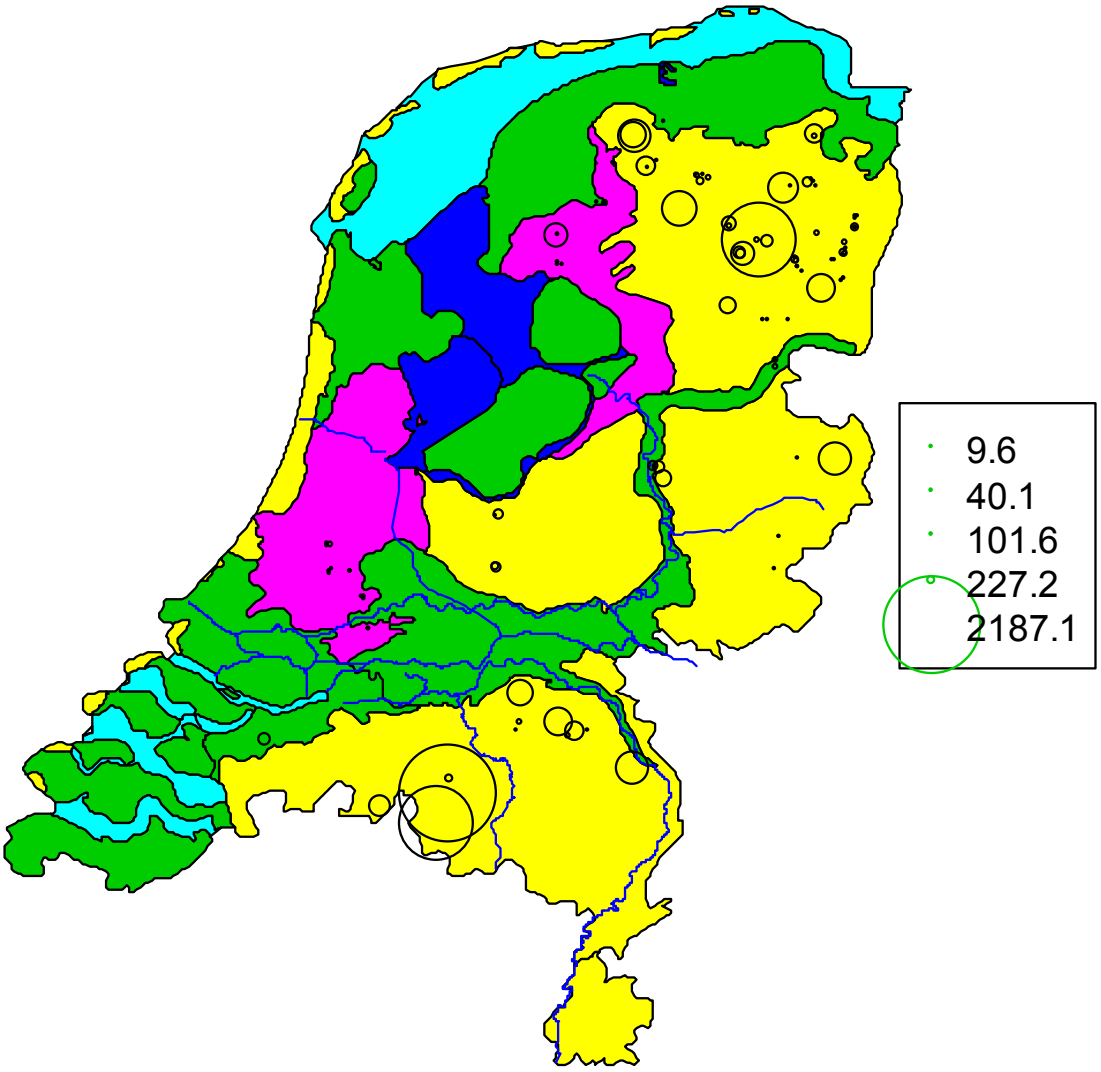


Figure 3: The half-life of the acetate mineralization at the groundwater table in the Netherlands. These are the raw values expressed in minutes without logarithmic transformation and not corrected for soil dilution in the samples. Note that the large circles in Figure 2 are now presented as very small dots.

3.2. The correlations between the different subsoil properties

Table 3 shows the correlation between the measurements. It is presented here and not at the end of the report where Table 1 and Table 2 are found. The logarithmically transformed mineralization rate is called "lograte". This "lograte" correlates with different subsoil properties which also correlate among themselves. On average "lograte" showed the strongest absolute correlation with the other subsoil properties. The second one is "dw" (the percentage dry weight). The brown peat subsoils typically had a high moisture holding capacity which resulted in a low percentage dry weight. Sampling was performed at the depth of the groundwater table and therefore the fresh samples were always soaking wet. The white sandy subsoils had a much higher percentage of dry weight.

Table 3: The correlation between the logarithmically transformed acetate mineralization rate (lograte) and the groundwater and subsoil properties. The Dutch coordinate system is expressed as y and x expressed in meters. The depth of the water table which is also sampling depth was expressed in meters below the surface. The electric conductivity EC was expressed in µS/cm. N is the nitrate concentration in mg/liter.

	y	x	depth	dw	EC	pH	N	lograte
y	1.00	0.69	0.13	0.28	-0.36	-0.19	-0.02	-0.28
x	0.69	1.00	0.37	0.67	-0.52	-0.47	0.23	-0.64
depth	0.13	0.37	1.00	0.40	-0.16	-0.41	0.31	-0.47
dw	0.28	0.67	0.40	1.00	-0.47	-0.54	0.34	-0.82
EC	-0.36	-0.52	-0.16	-0.47	1.00	0.35	0.02	0.39
pH	-0.19	-0.47	-0.41	-0.54	0.35	1.00	-0.50	0.59
N	-0.02	0.23	0.31	0.34	0.02	-0.50	1.00	-0.50
lograte	-0.28	-0.64	-0.47	-0.82	0.39	0.59	-0.50	1.00

3.3. Correlation between acetate mineralization and soil properties

The acetate mineralization was compared with the percentage dry weight and the coordinates of the subsoil from the sites and with the pH, conductivity, nitrogen content of groundwater from the sampling sites. Table 3 shows the correlation between these measurements. The highest absolute correlation was found between lograte and the percentage dry weight. Another strong correlation was found between the log rate and the x coordinate. The peat samples have a low dry weight because the wet peat samples did contain a large percentage of water. Negative correlations in the dry weight (dw) column of Table 3 correspond with positive correlations in the lograte column and vice versa. These correlations confirm the first impression that one gets from Figure 2 and Figure 3 that it are the peat soils that show higher mineralization rates. Note that the sampling depth in our experiments is at the upper groundwater level where both oxygen from the air and the groundwater are present. The mineralization rate in the lower anaerobic part of the groundwater in peat soils is very low because of the low pH combined with the lack of oxygen. Table 3 indicates a weak correlation between the the depth and the logarithm of the acetate mineralization rate. As expected from the literature (Van Beelen et al., 1994) a greater depth exhibited a decreased mineralization rate. The correlation is however rather weak.

Figure 4 shows the correlation between dw (meaning peat soils at the left-hand side and sand soils at the right) and the groundwater depth, the pH, the nitrate concentration N in milligram nitrate/liter and the electric conductivity EC. Most peat soils had a groundwater level less than 1 meter deep. Which was expected because these soils are in the lower parts of the Netherlands. The peat soils did not show a low pH as one might expect from natural peat soils. These peat soils are used as pastures and are therefore amended with chalk to raise the pH to values between 6 and 7 to optimize the growth of the grass. The nitrate content

in the peat soils was generally low. This is probably caused by incidental anaerobic conditions after heavy rains. Anaerobic conditions are necessary for denitrification which removes nitrogen (Van Beelen and Doelman, 1997). The electric conductivity in the peat soils was often relatively high. This might be explained by their closer proximity to the sea. In these low areas close to the sea brackish groundwater can come up to the surface. These soils also have a low x coordinate because they are in the lower Western parts of the Netherlands.

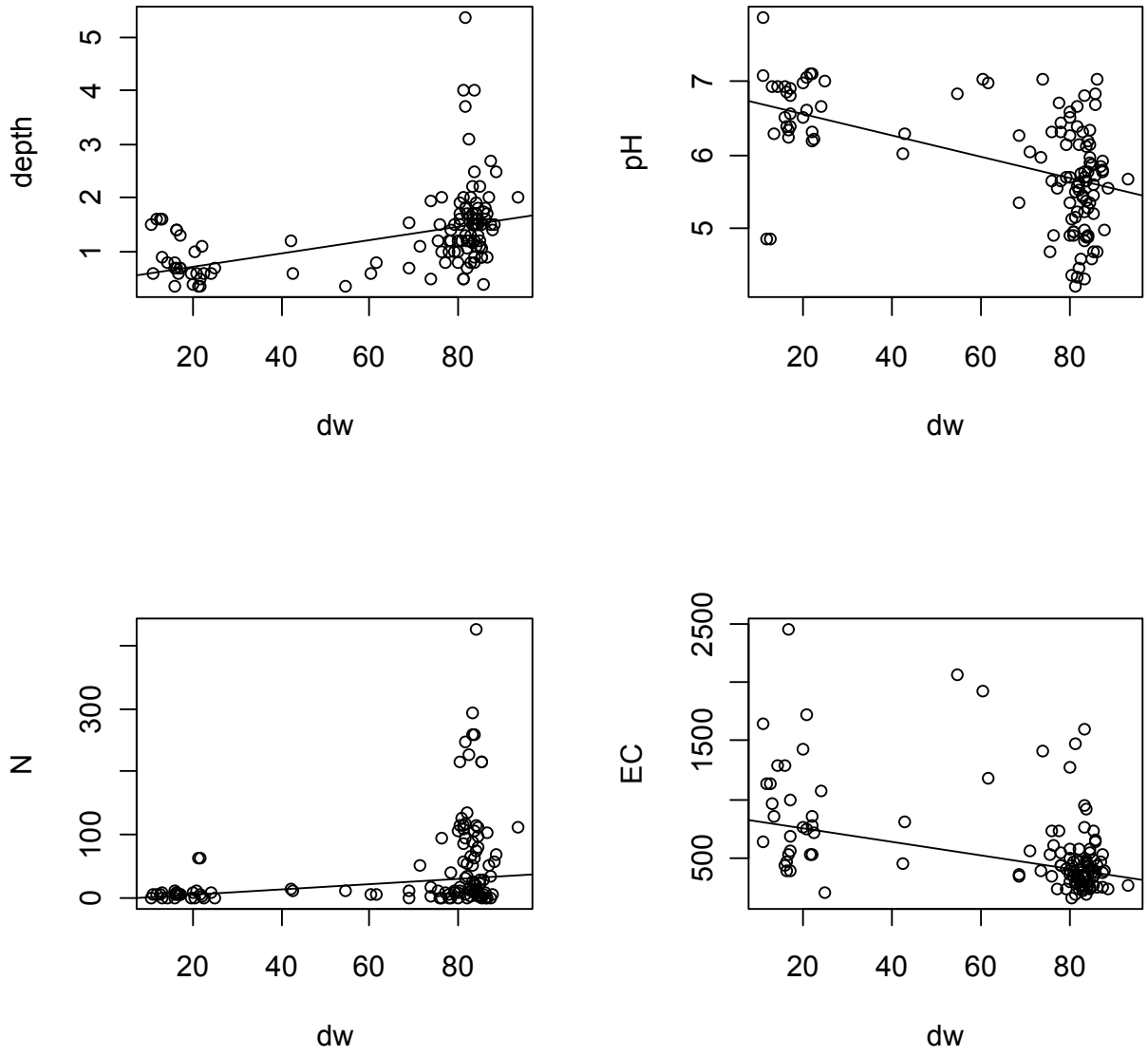


Figure 4: The correlation between the percentage dry weight (dw) with the groundwater depth (meters), groundwater pH, the nitrate concentration N (mg nitrate/liter) or the electric conductivity EC (µS/cm). Peat has 20% dry weight whereas sand has 80%.

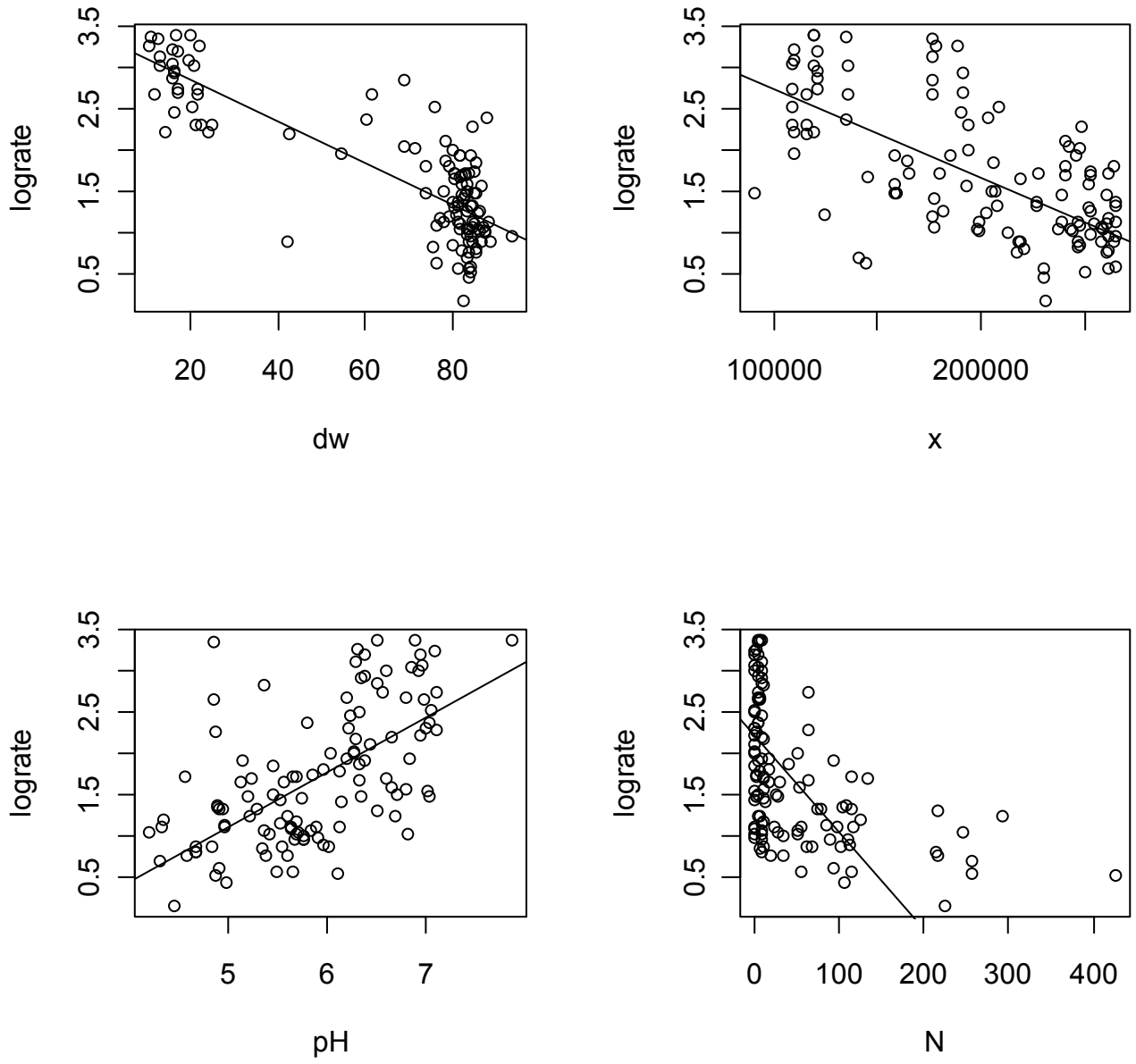


Figure 5: The correlation between the logarithmically transformed acetate mineralization rate (lograte) and the soil properties; percentage dry weight (dw), West East coordinate (x), groundwater pH and milligram nitrate/liter (N).

Figure 5 shows the correlation between the logarithmically transformed acetate mineralization rate (log rate) of the subsoils with the soil properties. Only the four strongest correlations from Table 3 are presented. The shown regression lines are just for illustration and do not mean that there is a linear relationship between the soil property and the lograte. In our samples and actually also in the Netherlands is a clear distinction between the peat soils (located around 20% dry weight) and the sand soils (around 80% dry weight). Drawing a straight line between two clouds of points will have a strong correlation but this does not mean that there is a linear relationship. The correlation between lograte and the soil factors is always rather weak. This indicates that one cannot estimate the self purifying capacity of these subsoils from their soil properties.

3.4. The self purifying capacity

Figure 5 indicates that the peat subsoils show a much higher (about 30 times higher) mineralization rate compared to the sandy subsoils. Actually a lot of peat soils are more active than the surface soils which show a lograte between 2.6 and 2.9 (Table 2). These surface soils were from a forest and a pasture listed at the end of Table 2. More important than the differences between the samples are the similarities. The mineralization rates ranged from 1.5 to 2500 per minute per kg soil (see sample 25 and 60 in Table 1). The acetate mineralization rates might be used as a starting point for the prediction of the biodegradation of other organic compounds using structure biodegradation relationships (Posthumus et al., 2005). This gives ample opportunity for biodegradation in Dutch subsoils. While the rate is lower the residence time in the subsoil is much larger than in soil. The residence time of water in soil can be a couple of months while the residence time of the upper groundwater can be in the order of decades (Van Drecht et al., 2003). Therefore the activity of subsoil bacteria contributes largely to the self purifying capacity. In environmental studies, peat soils are generally considered less vulnerable to groundwater pollution compared to sandy soils. This is traditionally attributed to the much higher sorption capacity of peat soils but this study shows that also the high self purifying capacity of of peat soils can play a positive role.

4. Discussion

4.1. The comparison of the half-life of acetate with previous studies

We have performed previous studies with subsoil slurries in which the acetate mineralization was measured with the older $^{14}CO_2$ method (Van Beelen et al., 1991). Since that method used the same substrate under the same conditions with a totally different technique a comparison is interesting. The maximal value for the half-life of acetate from Table 1 (presented at the end of this report) is approximately 600 minutes. These values were measured at 20 °C instead of the groundwater temperature of 10 °C. Assuming that the half-life doubles with each drop of 10 °C in temperature, the half-life would become 1200 minutes that is about 0.83 day. The half-life of 0.83 day is within the range of the half-lives of 0.5 and 2.5 days measured for acetate at 2.5 and 3.5 meters deep in a white sand subsoil under a sheep meadow in Bilthoven (Van Beelen and Fleuren-Kemilä, 1993). In these studies also a slurry with equal amounts of fresh soil and water were used. In other white sand subsoils half-lives of 0.7 and 1.8 days were measured at 1.5 meters deep using the older $^{14}CO_2$ method (Van Beelen and Fleuren-Kemilä, 1993). Longer half-lives (up to 18 days) were reported when samples were taken deeper under the groundwater table using a bailer boring (Van Beelen et al., 1991). The bailor boring is a special technique which allows to take samples under the groundwater table. It is important to stress that the present study is limited to the self purifying capacity at the upper aerobic part of the groundwater. Our mineralization rates should not be extrapolated to anaerobic parts of the groundwater.

4.2. The derivation of threshold values for the groundwater directive

The study indicates that there is a large and reliable potential for biodegradation in the upper parts of the groundwater in the Netherlands. The method described here can be used when threshold values would be derived for specific organic substances. Additional experimental studies are then needed with these specific organic substances because extrapolation of the mineralization rate from one substance to another is not yet reliable enough. Up to now no threshold values are being derived for organic substances in the Netherlands. At this moment the focus is on inorganic substances which cannot be mineralized (Verweij et al., 2008).

5. Conclusions

The newly developed method to measure the ecosystem service acetate mineralization was easy and reliable. The acetate mineralization rate at the groundwater table in Dutch peaty subsoils is often more rapid compared with surface soils from a meadow or a forest. In sandy subsoils the rate can be two orders of magnitude lower than in the surface soils. Nevertheless, the self purifying capacity of the subsoil can be vital under conditions that the surface soil is inactive because of freezing, drought or during incidental flooding. The self purifying capacity of subsoils is therefore very important for groundwater quality.

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Table 1: The half-life of acetate calculated from the scintillation data. Sample 10 is shown in Figure 1 as an example.

	Half-life	*std.error*	*t.value*	*maxipercent*
1	127	18	7	80
2	232	39	6	92
3	123	13	10	75
4	75	12	6	62
5	214	27	8	89
6	144	11	13	89
7	60	6	11	83
8	16	4	4	59
9	141	16	9	85
10	90	13	7	80
11	15	2	8	85
12	81	12	7	87
13	141	21	7	36
14	290	47	6	43
15	139	40	3	30
16	67	8	9	32
17	64	5	14	39
18	77	8	10	33
19	29	2	15	28
20	90	6	16	36
21	43	3	15	40
22	245	50	5	48
23	36	4	10	33
24	101	12	8	40
25	571	112	5	78
26	104	8	14	56
27	69	7	9	54
28	104	11	9	59
29	108	12	9	63
30	226	19	12	64
31	77	6	12	64
32	22	2	9	44
33	215	40	5	62
34	62	10	6	43
35	61	5	12	58
36	40	6	7	62
37	10	5	2	4
38	33	11	3	7
39	31	7	4	11
40	58	28	2	2
41	25	10	3	37
42	65	9	7	61
43	45	11	4	50
44	27	7	4	26
45	17	3	5	9
46	35	12	3	3

	Half-life	*std.error*	*t.value*	*maxipercent*
47	10	2	6	14
48	10	1	7	72
49	73	11	7	14
50	60	9	7	37
51	68	10	7	50
52	38	5	8	36
53	102	11	9	52
54	147	33	4	85
55	36	5	7	49
56	15	2	7	54
57	85	9	10	75
58	33	3	13	71
59	25	2	16	70
60	27	2	16	48
61	569	152	4	80
62	51	8	6	56
63	143	24	6	91
64	58	8	8	34
65	26	3	8	48
66	106	19	5	60
67	29	3	9	44
68	27	4	7	65
69	40	6	6	39
70	25	3	8	59
71	14	1	14	69
72	17	1	12	55
73	20	4	5	53
74	21	3	7	58
75	18	4	5	9
76	11	2	5	31
77	21	5	4	12
78	11	1	7	56
79	21	7	3	55
80	20	2	8	73
81	773	156	5	85
82	156	19	8	76
83	1651	260	6	87
84	385	35	11	81
85	722	155	5	87
86	301	24	13	82
87	625	108	6	86
88	147	19	8	80
89	100	11	9	82
90	464	59	8	86
91	453	54	8	86
92	308	24	13	84
93	272	30	9	70
94	96	9	11	78

	Half-life	*std.error*	*t.value*	*maxipercent*
95	166	16	10	63
96	263	24	11	83
97	1653	493	3	87
98	676	135	5	79
99	168	20	9	77
100	121	13	9	63
101	283	39	7	75
102	102	10	10	68
103	374	26	14	70
104	94	22	4	70
105	770	156	5	81
106	183	25	7	80
107	173	50	3	80
108	221	31	7	55
109	194	51	4	67
110	44	4	11	69
111	33	5	7	65
112	94	18	5	49
113	17	2	7	46
114	69	6	13	68
115	28	2	12	28
116	170	27	6	51
117	389	42	9	80
118	817	218	4	79
119	772	203	4	77
120	2187	283	8	80
121	116	16	7	58
122	652	112	6	79
123	530	91	6	77
124	161	26	6	62
125	270	50	5	66
126	607	116	5	81
127	139	17	8	65
128	468	84	6	66

Maxipercent is the maximal percentage of acetate still present at the shortest incubation time. The half-lives with a corresponding maxipercent <10% are unreliable because most of the acetate was already mineralized at the shortest incubation time.

Table 2: The acetate mineralization rate expressed in mL/(min*kg) subsoil together with the data from the sampling of the wells.

X	y	Depth	Date	lograte	dw	EC	pH	N	Crop	no	code
221028.9	569330.44	0.9	20-03-2008	0.8	85	730	4.7	215.0	arable	1	A7
230395.08	546360.13	4	24-04-2008	0.6	81	180	5.5	56.0	arable	2	B10
247366.32	542647.3	1	09-04-2008	0.8	80	300	5.4	7.0	arable	3	C3
247633.59	542916.02	1	09-04-2008	1.1	76	340	5.6	0.0	arable	4	C6
246953.29	544870.56	1.2	10-04-2008	0.9	42	450	6.0	12.0	arable	5	C14
261390.16	546397.4	1.2	08-04-2008	0.8	82	318	5.6	35.0	arable	6	D6
238856.4	527014.58	1.5	31-03-2008	1.1	88	382	5.0	55.5	arable	7	E8
261234.7	539105.05	0.8	26-03-2008	1.7	84	190	5.7	10.5	arable	8	F13
261028.13	550071.78	1.5	16-04-2008	0.8	84	456	5.4	18.5	arable	9	G2
253143.78	566326.64	1.7	19-05-2008	1.0	83	330	5.8	89.5	arable	10	H15
264379.97	557160.73	0.5	02-04-2008	1.8	74	390	6.0	17.0	arable	11	J4
264848.74	554412.79	2	11-04-2008	1.0	93	258	5.7	111.0	arable	12	K6
217610.68	569661.54	0.9	20-03-2008	0.8	85	380	4.6	215.5	arable	13	A16
230682.14	546694.12	4	24-04-2008	0.5	84	360	5.0	107.0	arable	14	B8
246983.94	544812.48	1.2	10-04-2008	0.8	76	520	4.7	9.5	arable	15	C4
247184.7	545273.37	1.2	10-04-2008	1.1	78	430	5.6	0.0	arable	16	C5
260931.2	546191.06	1.2	08-04-2008	1.1	85	235	6.1	23.0	arable	17	D14
244574.31	527221.44	1.5	31-03-2008	1.0	88	365	5.8	34.0	arable	18	E16
260573.92	538305.02	0.8	26-03-2008	1.5	83	260	5.7	11.0	arable	19	F1
261790.71	547896.77	1.5	16-04-2008	1.0	84	356	5.8	8.5	arable	20	G7
251837.44	568117.93	1.7	19-05-2008	1.3	80	490	6.5	216.0	arable	21	H5
250438.51	567694.86	1.7	19-05-2008	0.5	84	920	4.9	426.5	arable	22	H9
265153.74	557470	0.5	07-04-2008	1.4	81	340	4.9	107.5	arable	23	J9
264425.96	553876.38	2.5	11-04-2008	0.9	89	236	5.6	69.0	arable	24	K14
231264.18	546919.05	3.1	24-04-2008	0.17	82	460	4.5	225.5	arable	25	B5
258236.21	544897.02	1.6	16-04-2008	0.9	85	540	6.0	112.5	arable	26	G10
257954.34	544613.83	1.5	16-04-2008	1.1	85	575	5.4	8.0	arable	27	G15
219459.66	569886.02	0.9	19-03-2008	0.9	86	440	4.7	102.0	arable	28	A2
217901.28	569629.98	0.9	20-03-2008	0.9	84	260	4.8	62.5	arable	29	A4
261434.39	546394.03	1.2	08-04-2008	0.6	84	483	6.1	257.0	arable	30	D5
237577.41	527109.28	1.5	31-03-2008	1.0	83	220	5.4	51.0	arable	31	E1
252047.63	567893.26	1.7	19-05-2008	1.6	82	350	6.6	52.5	arable	32	H1
264868.11	554271.09	1.6	11-04-2008	0.6	84	468	5.7	114.0	arable	33	K1
264802.27	554170.77	1.8	11-04-2008	1.1	85	397	5.9	97.0	arable	34	K2
261409.44	538878.97	0.8	26-03-2008	1.2	77	230	5.5	8.0	arable	35	F10
264912.36	557297.56	0.5	07-04-2008	1.3	81	460	5.0	115.5	arable	36	J10
121142.16	435779.62	0.7	04-07-2008	2.9	16	390	6.4	5.0	pasture	37	M3
108847.7	460686.18	0.35	08-07-2008	2.3	21	530	7.1	63.5	pasture	38	N8
115561.99	452779.55	0.6	11-07-2008	2.3	22	720	6.2	0.0	pasture	39	P11
109648.35	452699.05	0.8	24-06-2008	2.2	14	1288	6.9	0.0	pasture	40	R13
176765.14	543679.21	1.6	12-06-2008	2.7	12	1130	4.9	5.0	arable	41	S14
177161.8	551785.96	1.2	13-06-2008	1.4	82	570	6.1	12.6	arable	42	T12
193146.44	572895.92	1.7	20-06-2008	1.6	86	249	7.0	0.0	pasture	43	U1
193588.76	572706.84	0.7	20-06-2008	2.3	25	200	7.0	0.0	pasture	44	U8
191196.1	561070.12	1.3	19-06-2008	2.7	17	560	6.8	6.0	pasture	45	V8

X	y	Depth	Date	lograte	dw	EC	pH	N	Crop	no	code
119275.07	445429.16	0.6	10-07-2008	2.2	24	1070	6.6	8.0	arable	46	W15
135051.46	482297.79	0.6	25-06-2008	2.4	60	1920	7.0	5.0	pasture	47	X6
135481.72	481825.68	0.9	27-06-2008	3.0	13	960	6.9	0.0	pasture	48	X14
121209.12	435538.27	0.7	04-07-2008	2.7	17	680	6.6	5.0	pasture	49	M2
121261.58	435545.71	0.7	04-07-2008	2.9	16	440	6.5	9.5	pasture	50	M6
115748.08	452809.9	0.5	11-07-2008	2.7	22	850	6.2	5.5	pasture	51	P5
109025.68	460059.19	0.35	08-07-2008	3.0	16	470	6.9	5.0	pasture	52	N16
109155.04	452291.99	1	24-06-2008	2.5	21	1720	7.0	0.0	pasture	53	R16
190699.56	561409.39	1.4	19-06-2008	2.5	17	2460	6.2	8.0	pasture	54	V12
178014.54	543203.75	1.5	12-06-2008	3.3	11	632	7.1	0.0	pasture	55	S9
176595.76	551961.26	0.7	13-06-2008	2.8	69	360	5.4	11.1	pasture	56	T2
193952.35	572670.38	0.8	20-06-2008	2.0	80	350	6.3	0.0	pasture	57	U15
119385.67	444955.83	0.6	10-07-2008	3.0	21	750	6.6	10.0	pasture	58	W4
135481.57	481638.16	0.8	27-06-2008	2.7	62	1180	7.0	6.0	pasture	59	X2
135387.55	481920.47	0.6	27-06-2008	3.4	11	1650	7.9	6.0	pasture	60	X3
176496.4	551815.25	1	13-06-2008	1.2	79	230	5.7	10.2	pasture	61	T3
191186.01	561385.9	1.4	19-06-2008	2.9	17	520	6.3	9.0	pasture	62	V6
109600.63	461089.92	0.35	08-07-2008	1.9	55	2070	6.8	10.0	pasture	63	N4
108847.7	460686.18	0.35	08-07-2008	2.7	22	530	7.1	63.5	pasture	64	N8
121142.16	435779.62	0.7	04-07-2008	3.2	17	390	6.4	5.0	pasture	65	M3
115485.06	452957.86	0.6	11-07-2008	2.2	43	800	6.3	10.5	pasture	66	P4
110265.7	453359.99	0.6	24-06-2008	3.1	20	1430	7.0	0.0	pasture	67	R7
109648.35	452699.05	0.8	24-06-2008	3.2	16	1288	6.9	0.0	pasture	68	R13
176813.12	543594.32	1.6	12-06-2008	3.1	13	860	6.3	9.0	arable	69	S5
176765.14	543679.21	1.6	12-06-2008	3.3	13	1130	4.9	5.0	arable	70	S14
119611.2	444306.47	0.4	10-07-2008	3.4	20	760	6.5	8.0	pasture	71	W1
119446.58	444962.02	0.6	10-07-2008	3.4	17	990	6.9	5.0	pasture	72	W12
199469	581582	1.6	2-7-2009	1.0	86	645	6.8	0.0	pasture	81	17912 - 1
227280	531229	1.3	12-5-2009	1.3	84	432	4.9	79.3	arable	84	62668 - 1
243778	565821	1.75	24-6-2009	1.0	86	366	5.7	27.0	pasture	85	35659 - 1
238781	550019	0.7	23-6-2009	1.4	82	237	5.5	0.0	pasture	86	96536 - 16
199450	581392	2	2-7-2009	1.1	81	388	5.0	86.0	pasture	87	17912 - 16
252585	581443	2.2	29-6-2009	1.7	85	406	5.9	1.7	arable	88	31229 - 16
245550	566630	1.8	23-6-2009	1.9	82	275	6.4	5.0	pasture	89	35659 - 16
181386	405900	1.5	12-5-2009	1.3	83	1606	5.2	292.3	arable	90	28645 - 1
252600	582292	1.8	30-6-2009	1.2	86	635	6.7	6.7	arable	91	31229 - 1
90560	402816	1.95	27-5-2009	1.5	74	1420	7.0	2.0	arable	92	98000 - 1
159146	469800	1.1	20-5-2009	1.5	85	251	6.3	27.0	pasture	93	81678 - 1
158587	468881	1.1	25-5-2009	1.9	84	419	6.2	18.3	arable	94	81678 - 16
165156	408434	1.5	11-5-2009	1.7	80	574	5.7	114.7	arable	95	29128 - 16
205076	484231	1.6	6-7-2009	1.5	83	232	5.5	26.0	pasture	96	37741 - 1
140824	378705	2.2	6-5-2009	0.7	83	945	4.3	257.7	arable	97	86453 - 1
177110	408697	2	26-5-2009	1.1	87	471	5.8	51.3	arable	98	30883 - 16
179696	404693	1.6	26-5-2009	1.7	80	1275	6.6	11.7	arable	99	30883 - 1
164778	405915	1.4	7-5-2009	1.9	78	441	6.3	40.0	pasture	100	29128 - 1
207099	483193	1	8-7-2009	1.5	78	734	6.7	3.7	pasture	101	37741 - 16
185660	405915	1.3	12-5-2009	1.9	81	290	5.2	93.3	arable	102	28645 - 16
227235	555470	1.2	21-4-2009	1.4	80	382	4.9	104.3	arable	103	20464 - 16

X	y	Depth	Date	lograte	dw	EC	pH	N	Crop	no	code
247906	486300	1.1	14-5-2009	2.0	71	554	6.0	51.0	pasture	104	90573 - 16
198576	394800	3.7	26-5-2009	1.0	82	1475	4.2	246.3	arable	105	86016 - 1
144955	391683	1.8	20-5-2009	1.7	82	339	5.6	29.3	pasture	106	12505 - 1
253271	553106	2	9-6-2009	1.7	83	343	6.3	64.7	arable	107	12461 - 1
158384	454277	1.15	7-5-2009	1.6	83	755	6.8	12.0	pasture	108	24862 - 16
218720	567765	1.9	11-6-2009	1.6	80	160	5.1	16.7	pasture	109	61539 - 16
248950	540914	1.6	30-4-2009	2.3	84	269	4.9	3.3	pasture	110	49797 - 1
203676	572120	1.4	3-6-2009	2.4	88	531	5.8	5.7	pasture	111	80067 - 16
242505	462695	1.55	12-5-2009	2.0	69	347	6.3	0.0	pasture	112	11039 - 1
188713	561433	1.1	8-6-2009	3.3	22	774	6.3	1.7	pasture	113	71273 - 16
240949	453215	1.2	14-5-2009	2.1	78	539	6.4	0.0	pasture	114	32335 - 1
208362	585139	1.5	8-6-2009	2.5	76	737	6.3	0.0	pasture	115	20505 - 1
240985	515326	1.05	27-5-2009	1.7	82	487	5.2	134.0	arable	116	32681 - 16
208084	479889	1.9	20-5-2009	1.3	84	297	5.3	75.3	pasture	117	59511 - 1
212622	559578	2.7	3-6-2009	1.0	87	255	5.9	0.0	pasture	118	98537 - 16
258584	486021	2.5	10-6-2009	1.0	84	348	5.7	8.3	pasture	119	58336 - 1
144493	387776	2	28-5-2009	0.6	76	613	4.9	94.0	arable	120	13667 - 1
206343	574219	1.5	29-5-2009	1.8	85	347	5.5	0.0	arable	121	54203 - 1
254652	536163	5.35	11-5-2009	1.1	82	318	4.4	116.7	pasture	122	18601 - 16
124721	383488	1.2	7-5-2009	1.2	81	437	4.4	124.7	pasture	123	28678 - 1
227336	555058	1.3	21-4-2009	1.7	83	293	4.6	3.3	pasture	124	20464 - 1
158384	454277	1.05	14-5-2009	1.5	85	269	5.2	2.3	pasture	125	25906 - 16
240800	513496	1.5	26-5-2009	1.8	79	398	6.1	6.7	pasture	127	32681 - 1
202981	572625	0.4	3-6-2009	1.2	86	486	5.6	4.7	pasture	128	80067 - 1
165769	416909	1.8	30-4-2009	1.2	81	x	5.3	34.7	pasture	126	27036 - 16
235114	550597	2.6	18-6-2009	1.7	88	x	x	4.0	pasture	82	79165 - 1
235904	550711	3	19-6-2009	0.7	88	x	4.4	61.7	arable	83	79165 - 16
				2.6	79				forest	73	HS_b1
				2.6	79				forest	74	HS_b2
				2.7	79				forest	75	HS_b3
				2.9	79				forest	76	HS_b4
				2.6	86				pasture	77	HS_g1
				2.9	84				pasture	78	HS_g2
				2.6	85				pasture	79	HS_g3
				2.6	85				pasture	80	HS_g4

dw = percentage dry weight of subsoil
depth = groundwater level in meters (also sampling depth)
EC = electric conductivity in µS/cm
N = nitrate content of the groundwater in mg nitrate/liter
x and y Dutch Amersfooort coordinates in meters

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