Supplemental Material

Biosolid and distillery effluent amendment to Irish *Miscanthus X giganteus* plantations: impacts on groundwater and soil

**MATERIALS AND METHODS**

*Teagasc:* The government organization denoted in the manuscript as “Teagasc” is the Irish agriculture and food development authority (for more information please refer to [http://www.teagasc.ie](http://www.teagasc.ie)).

*Site geology:* The geology of the Oak Park site is one of limestone-drift materials overlying regional limestone-bedrock (over which two broad groups of soils occur: light-textured gravelly soils derived from limestone, and more heavy-textured soils derived from till). The soil underlying *Miscanthus*-plantations used in the experiments the former type, with a perched clay/subsoil layer under the BS-application site.

*Soil-characteristics, hydrology:* Previous studies in Oak Park have established vertical travel-rates through soils, horizontal-travel through the GW aquifer and the effective soil porosity and $K_{sat}$ values for soil-profiles (and groundwater aquifer). The vertical pore-velocity of soil is 15.7 mm day$^{-1}$, which corresponds to an “effective porosity” ($n_e$) of 25% through the unsaturated-zone to the groundwater-aquifer (Premrov et al., 2007). This reflects the nature of the light soils on-site, with the gravel-sediment layer coming close to surface topsoil in several areas (including the
“Barley Field” area containing the *Miscanthus* plantation where DE was applied. A Br-tracer experiment conducted by Hooker (2005) provided daily travel-times for N through the vertical profile of 0.02 to 0.03 m day^{-1}. The breakthrough of Br-tracer occurred at a depth of 0.9 meters between 27 to 42 days following surface-application of the Br-tracer to the soil-surface, this is in agreement with EC breakthrough-times for meter flow-through columns (which showed peaks at 31.5 days on average for the soils from *Miscanthus* plantations). Fenton *et al* (2009) looked at travel-times through the sand- and gravel-type aquifer under the *Miscanthus* plots: the $K_{\text{sat}}$ values for the aquifer were between 5-100 m day^{-1} while the flow velocity ($v$) was between 0.244 to 4.88 m day^{-1}; resultant horizontal travel-times were 5.6 to 0.28 years (to a drain-point approximately 500 meters away. The lake is within 500 meters of both plot-sites, while the River Barrow is approximately 2,000 m from the *M. giganteus* plots.

**Groundwater depths:** The depth to GW is measured from soil-surface to the surface of the water in the aquifer, and is given in meters (m). The average figure for GW depth (for all plots) was 2.77 m (a 12-month average) with a seasonal-variation (December to June) of 43 cm.

**Climate conditions:** Ireland has a temperate, mild, wet climate dominated by Atlantic weather systems with year-round precipitation and soils that rarely dry out. Data on climate-conditions was obtained from Met Eireann’s (the Irish national metrology service’s) synoptic weather-station in Oak Park. The metrological station referred to is located approximately 500 meters (to the NE) from both amendment-sites, and is located within the boundaries of the research station, being reported as the “Oak Park,
Carlow, Synoptic Station” in weather-reports in Ireland (which are available at [http://www.meteireann.ie](http://www.meteireann.ie)).

Average temperature and rainfall during the experimental period (October 2007-October 2009) were above 30-year averages (1960-1990). Annual rainfall was 906mm during the experimental period, while the effective rainfall-rate during the experimental period was 331.1mm. Rainfall in the summer months was high for the experimental period (2007-09), with average rainfall for June (105.2mm) being higher than January (87.9mm).

**Crop yields:** Annual yields and crop-moisture were established using Teagasc guidelines (Coulter and Lawlor, 2008). Mean annual-yields for Miscanthus were between 5.5 to 7.7 t ha$^{-1}$ dry wt; the estimated yield under optimum Irish-conditions is 10-13 t ha$^{-1}$ dry wt. Crop-moisture prior to harvesting was 20-35% (w/w). Crop was harvested annually from 2006 (previously the crop was left fallow).

**Site characteristics for DE$_x$ plots:** The mean depth of DE-site GW was 1.9 m rising an average 1.55 meters from the lake-surface, representing a hydraulic gradient of 0.0045. The DE-site soil-profile comprised a shallow topsoil-layer (10 cm) and a secondary sandy subsoil layer (30 cm). The subsoil-layer contained several sub-layers (1-2 cm) of stones at intervals of 10 cm. Subsoil overlay a layer of gravelly sediments (1.4 m) containing the saturated GW layer. The DE-site soil had a bulk density of 1.48 g/cm$^3$, a (topsoil) organic matter content of 5.8% (w/w) and a stone content of 20.5% (w/w). The mean summer soil-moisture content (June) was 26.4%.
Average Miscanthus-yield from the DE-site was 5.32 t ha$^{-1}$ dry wt, at a crop dry-
matter (DM) content of 18%.

Site characteristics for BS$_x$ plots: The mean GW-depth under the BS-site plots was
3.1 meters, rising an average 1.42 meters from the lake-surface, representing a
hydraulic gradient of 0.0067. Beneath a shallow topsoil layer, the BS-site soil-profile
incorporated a significant clay sub-soil layer (1.2 m) overlaying a sand/gravel
sedimentary layer (1.9 m) (similar to the DE-site) which contained the GW. Soil-
stratification at the BS-site was the A-B-C sequence typical of Podzols in which the
topsoil layer (A) is the zone of biological activity, subsoil (B) comprises intermediate
materials and the (C) horizon is made up of parent material (Hillel, 1983). Soils
underlying the BS-site had a mean density of 1.66 g/cm$^3$, a topsoil organic-matter
content of 8.3% (w/w) and a stone-content of 14.7% (w/w). The mean summer (June)
soil-moisture content was 28.2% (w/w). Crop-yield was 7.82 t ha$^{-1}$ dry wt. with an
average DM content of 34.1% (at harvest).

Additional information on plots: The distance between plots in the DE-site varies as
plot-placement-pattern was not in a regular formation. The DE$_{50}$ and DE$_0$ plots were
approximately 10 meters apart; while the MDE$_{100}$ plot was set back roughly 30 meters
from these two. All BS$_x$ plots were arranged in a line, a minimum of 15 meters apart.

Cross-contamination: There is a possibility the circuitous pathways involved in
advection and diffusion through soil could cause cross-contamination, though given
the physical separation of the plots, it is most likely to be a marginal, edge-like effect.
The plots with 100% treatments were at lower points on the hydraulic gradient, and
were separated by a considerable distance from the controls, making cross-contamination unlikely. If such cross-contamination did occur, the placement of multiple-wells should also help to ensure any short-lived cross-contaminating plumes do not overwhelm the entire plot-area; longer lived plumes would be more problematic. A plan-view map of the site is provided in the main text (Fig. 2).

*Organic by-product application, maximum limits:* The application maxima in Ireland are based on the soil-quality status and the approximate amount of nutrient that will be made available to the crop, defined by the “soil index”, Index 1 = V. low soil-P (0-3 mg l⁻¹), Index 4 = very high (or > 10 mg l⁻¹). In the Irish soil-nutrient classification system, “V. Low” relates to an available-to-crop soil N-level below “Index 1” this being the best of four soil-quality levels, with “Index 1” being the best and “Index 4” being the worst. It would indicate there is no excess of soil-N allowing an equivalent of 180 kg N ha⁻¹ to be spread, if the soil was classified as “Index 4”; this would only allow 75 kg N ha⁻¹ to be applied.

There is no accurate Irish laboratory-test for available soil-N; therefore when determining N for cropping-requirements, available soil-N is deduced from previous cropping and fertilization history together with soil-type. In this system, account is taken of previous applications of chemical fertilizer and livestock manure-N, the requirement of the crop, and the likely crop-yield. This will allow the farmer to determine what amount of fertilizer (if any) needs to be added in order to obtain the optimal nutrient/yield balance, while minimizing possible run off or leaching (from excess).

*Variation of Index values by nutrient:* Soil Index values are different for each applied nutrient, therefore a site will have set of values (i.e. for N, P and K) and these will
denote (separately) how much specific N, P, and K may be spread (e.g. both sites had a soil-P at “Index 4”, which indicates a large excess, allowing an application of 0 kg P ha⁻¹ (which is why spreading was limited by crop-uptake of P). In these experiments, the Soil Index values for Maize were used as there is no current set of Irish index values for Miscanthus (Maize was used as it is currently the closest equivalent C4 crop on the system) (Coulter and Lawlor, 2008).

*Limit of spreading defined by soil P Index:* The Index 4 status of background soil-P indicated that the site had high background soil P; fertilizer-borne P could only be added based on the potential uptake by *M. giganteus* (at a theoretical yield of 10-15 t ha⁻¹). Using these criteria, the higher end of potential P-uptake scale is 15 t P ha⁻¹; this was subsequently taken as the maximum that could be tolerated and this is represented by the 100% limit. Based on this system, the “50%” limit is therefore 7.5 t P ha⁻¹.

*Other amendments:* There were no commercial fertilizers (or amendments of any kind) spread on any Miscanthus-plots during the 2006-2009 experimental period. Additional water was not supplied to crop in summer months (this would be rare in Irish agriculture in any case). The crops were contained within long-existing stands (1993-present), and no replanting was conducted or required during the experimental-period; there were annual harvests from 2006 onward. Outside of OW-amendments and necessary maintenance of experimental equipment, site-markings, berms, plots no other interference occurred on either site.
Definition of regulation pertaining to spreading of organic by-product in Ireland:
The spreading of BS by-product (and by extension similar by-products, such as DE) are dealt with in Irish regulation SI No.148. The term “S.I.148” refers to the Irish Government Statutory Instrument No.148 of 1998 (DEHLG 1998); the S.I. is a legal device that brings Irish parliamentary legislation into force within the jurisdiction of the Republic of Ireland. Statutory instruments are legally defined as "an order, regulation, rule, scheme or bye-law made in exercise of a power conferred by statute." They were instituted by the Irish Statutory Instruments Act (1947).

Outline of Irrigation system: The irrigation-system (Fig. 1) comprised a PE pipe-network linked to a 9 m³ storage tank pumped by a 2.2 kW centrifugal pump (SV-1602-F22M. Lowara ITT. Lombardy, Italy). The network comprised a main pipe (O.D. 63 mm) running 280 m and six 42 m branch pipes (O.D. 50 mm) with three branch-pipes running onto each (treated) plot. Each branch incorporated four risers (2 m) topped by a Rainbird Falcon nozzle (Rainbird, Glendora, CA). Losses arising from irrigation were negligible though high-winds occasionally caused spray-drift. Use of the system was problematic due to coagulation of DE and the system had to be cleaned out on a regular basis, the DE was periodically re-circulated and aerated using a farm vehicle.

Groundwater wells: In terms of the operation of the wells, each bottom section had 3 mm slit-like inlets to allow the ingress of GW. A gravel-layer placed between the tube-wall and bore-hole wall filtered this ingressing GW. A nylon sock (10.01.04.01. Eijkelkamp. Giesbeek, Holland) placed over the bottom-section filtered the particulate
fines; this area was sealed with Bentonite to prevent ingress of soil-moisture from above.

*Groundwater level determination:* Groundwater-levels were determined using a depth-gauge (11.03.40. Eijkelkamp. Giesbeek, Holland). All depths being referred to are the depths to the GW aquifer (as measured from the surface of the GW) relative to the soil-surface at the point of measurement.

*Collection of GW samples:* Groundwater samples were collected in 250 ml, screw-cap PE sample containers from Sarstedt (75.9922.519 Sarstedt, Nümbrecht, Germany). These containers were used from new; each container was only used once to ensure they could not cross-contaminate samples. All samples were logged and kept in sterile 250 ml PE containers (79.9922.519. Sarstedt AG. Nümbrecht, Germany) and refrigerated below 5°C before being sent to the laboratory (usually within 7 days). Samples were tracked and recorded at each stage of collection and their processing in the Johnstown Laboratory, via the Teagasc Lab Information Management System.

*Groundwater considerations:* The level of groundwater in the saturated zone is not restricted by a clay lens under the DE\textsubscript{x} site (the “Barley Field”); however, there was a clay lens under the BS\textsubscript{x} site (the “The Near Avenue Meadow”). The saturated soil-layer containing GW in Oak Park consists of sedimentary-sands and gravel (derived from underlying limestone bedrock and glacial till). In some areas, the saturated layer approached the land-surface (usually during winter months) resulting in surface-pooling on the Oak Park site, though not on any sites containing *Miscanthus*-plantations.
Soil sampling: All soil-sampling procedures were conducted in accordance with Irish Regulation. Topsoil samples were taken at a depth of 25 cm, each top-soil sample was a composite of six sub-samples. To obtain four bulk samples per plot, a total of 24 sub-samples were taken in a “W” pattern, this sampling scheme was used for all treatment-plots using the same “W” pattern. There were samples taken at deeper depths from some plots, (or soils nearby) to characterize soils. These deeper soil samples were obtained during the boring of GW well holes (1-5 meters) and from soil-horizons revealed in soil-pits sunk onsite. Composite samples were taken from each identified soil-layer. All bulk soil-samples were immediately boxed in waxed soil-sample boxes, the samples were numbered and recorded and kept in refrigerated conditions (below 5°C).

Crop samples: Composite crop-samples were collected by removing (at least) 5 plants randomly from each plot. The plants were cut into small pieces and fully mixed to ensure a representative bulk-sample. The sample was weighed and dried, its moisture-content determined and a suitable amount (approx 500g) placed into large PE container bags (which were all numbered and recorded). The crop-samples were stored under 5°C before being sent to FBA Laboratories for analysis. Both the in house Johnstown Laboratory and commercial laboratory (FBA Labs) used for crop, soil and by-product sample analyses are fully accredited.

Additional information on sample analysis:
Groundwater analysis: Analysis of groundwater EC was conducted on-site using a TDS conductivity meter (ECW-1383A. TDS. Shanghai, China); pH with a Radiometer pH meter (PHM 82. Radiometer SAS. Lyon, France) and Schott Coaxial Electrode (L1 EE. Schott AG. Mainz, Germany).

Procedures for soil analysis: Once sent to the laboratory, soil samples were dried at 30°C and then ground and sieved through a 2mm mesh. For the determination of heavy metals (Cu, Cd, Cr, Ni, Zn, and Pb) 0.25g of dried sample was mixed with 9 ml of 70% HNO₃, trace-analysis grade and digested by microwave in closed vessels. Digests were made up to 100 ml with de-ionized water D.I. (18 MΩ resistivity). Extracts were analyzed by Agilent 7500 ce ICP-MS (Agilent Technologies, Santa Clara, CA), using a 5-point calibration curve. CRM 7001- Light Sandy Soil (Czech Metrology Institute) is used as quality control.

For soil-pH; 10ml of dried soil-sample was mixed with 20ml of de-ionized water (15 MΩ resistivity), allowed to stand for 10 minutes, and tested by Metrohm pH meter (Model 789) using a Refex pH - Glass membrane electrode. If the pH of the sample was greater than 6.5, no lime was required; if pH was lower than 6.5, 10ml of dried soil-sample was mixed with 20ml of SMP Buffer (Shoemaker, McLean & Pratt) at pH 7.5 and allowed to stand for 30 minutes. A set of in-house QC samples was used to check system performance. The pH value was read using a Metrohm pH meter Model 730, using Refex pH - Glass membrane electrode (DAF, 2004). For soil P&K 6 ml of dried soil-samples was mixed with 30ml of Morgan’s solution (Sodium Hydroxide and Acetic Acid) and shaken for 30 minutes. The extracts were filtered and the filtrates are analyzed, using a LACHAT 8000 series Flow Injection Analyzer (Hach Corp., Loveland, CO). In-house QC sample was used to check system performance.
Statistical Methods: It is considered inadequate to have two replications for the conduct of an experiment in the usual mode of identifying cause and effect; this arises primarily because of the follow-on difficulties in the accurate estimation of the mean and variance of the set of data obtained. The issue of the effect-size detectable from plots is probably a secondary issue, given these difficulties. The formal statistical analysis is to be interpreted in a purely exploratory sense; i.e., primarily providing information to inform future work rather than drawing firm conclusions from the present data-set. The aim is to highlight associations in data that will confirm or reject expectations but not assert cause-and-effect. The aim of using a lower level of evidence than the standard 0.05 $p$-value is not to correct the inference for the effects of limited replication but to allow interpretation of any reasonable level of evidence of association between the measured results and the factors in the experimental design. This is similar to the use of $p < 0.1$ in the selection of variables in a regression context. The aim is not to overlook any evidence of possible associations.

DISCUSSION

Potassium: Although ubiquitous in soil and important for plant-nutrition (due to its role an enzyme-activator) K is not considered a major pollution-concern; it is not prone to leaching from soil and its presence in wastewaters is not known to have any significant adverse health-effects (Arienzo et al., 2009). Limits have been established for K in GW and SW by the EU however, no toxicological justification currently exists for these limits, which appear to be mostly precautionary. Due to its ubiquitous
presence, K is not a limiting nutrient (unlike N and P) in the eutrophication of surface-water bodies and is not toxic to aquatic life. The main concerns regarding K relate to either deficiency in plants or animals, and/or imbalances between increased levels of K and other major nutrients in soils, having effects on the health of grassland and tillage-crop and resulting occasionally in dairy-cow health problems (Kayser and Isselstein, 2005).

There was percolation of K to GW; however, mean concentrations did not exceed GTV for any plots (Table 3) despite high background soil-K rates for all plots (Table 1). Several monthly-peaks exceeded the current K-GTV, with the highest peak occurring in July 2009 on BS$_{100}$ (Table 3). The mean K-concentration on BS$_{100}$ attained 80% of its respective GTV and it is possible that larger amendments could produce GTV breeches in averaged annual values. Distillery effluents contain K-salts and significant amounts of K$^+$ cations, but landspread-DE does not appear to impact soil-properties or influence K-loss (Arienzo et al., 2009).

It may be that added K was attenuated in soil-profile, as found in an Indian study (Kolahchi and Jalali, 2006) which may have been obscured by high background levels (Table 1). These authors also report increased K$^+$ leaching from sandy soils in the presence of Ca$^{2+}$ cations (Kolahchi and Jalali, 2007); however, Ca levels in GW and soil were not determined here. There was no evidence of excess-K in GW, indicating attenuation of K or possible loss to surface-flows. There was an increase in topsoil-K on DE$_{100}$, unlike control, (Table 4) indicating DE amendments impacted soil, but did not affect GW concentrations. It could be the case that fertilizer-borne K was not added at rates high enough to impact percolation; there was no build-up of K in GW
during the experiment for any plots (Table 4). Potassium-offtake rates may have played a role; it was found that crop-K concentrations increased on OW-amended plots during the course of the experiment (103%) with the greatest increases observed on maximum OW-amendment rate plots (on average 21% higher than controls). As with other nutrients it is possible K-losses to SW occurred; however, this aspect of K-loss is not dealt with here.

The occurrence of macropores, and potential macropore flow: The majority of soils of Oak Park are G/B Podzols containing clay-layers prone to macropore- and fissure-formation in dry conditions under certain cultivation-conditions (Gardner and Radford, 1980). Hooker conducted investigations into the occurrence of macropore-flow (and the existence of macropores) on the experimental-site during work conducted to assess nitrate-leaching from treated-crops (Hooker, K.V. 2005). Ryan (2010) looked at the issue of macropore-flow in Oak Park soils and identified the occurrence of both macropores and macropore-flow on several sites. Transport through macropores will be rapid, but associated travel-times to the saturated-zone containing GW depends on conditions, and the length and clarity of the pathway of a specific macropore to GW. Study on the possible movement of *E. coli* through macropores in Oak Park soils has identified the presence of macropores onsite (Brennan et al., 2010).

Additional discussion on soil pH decreases: The drop on low-amendment BS plots may have been due to variability as they were relatively small (-3% to -6%). If there was a statistically-significant drop, the phenomenon may have occurred for all plots regardless of treatment (which may have masked a small drop on BS_{100}). The
increases on DE plots were more pronounced; in all cases there were no additional amendments made to soils of BS or DE plots that would account for changes in pH. Changes in soil-pH may have arisen as a result of harvesting Miscanthus in 2006 (previously crop had been fallow for several years) resulting in improved uptake; which may have led to changes in soil-conditions (this is circumstantial and a causal link is not postulated). It is interesting that the pH-changes involved decreases in BS plots and increases in DE plots, given the presence of different soil-conditions (i.e. subsoil clay on BS). Changes may have occurred though cross-contamination of amendments across the surface, though for the BS plots this would not explain why treatments increased the soil-pH on the 100% plot. Any changes (outside of natural variability) could have occurred via variation in microbial-activity in the rhizomic zone. There were long periods of anaerobic-conditions in damp saturated top-soils on all plots. The drop in pH may be due to outside factors, such as atmospheric deposition precipitation with reduced pH etc, (which may have been masked by OB treatments).

Comparison of rainfall and percolation through soils: In terms of the overall climate situation, increased rates of precipitation were noted for all years (2007-2009) of the experimental-period; therefore it is not possible to make a meaningful comparison with other periods of more typical rainfall patterns. Investigation into links between monthly rainfall-rates and GW-P concentrations were conducted; however, correlation and covariance analyses were inconclusive. More in-depth statistical work on this aspect of the dataset (including other species) will be conducted in future. It is interesting that several recent papers indicate that climate-change impacts (such as
increased precipitation) may affect groundwater quality as well as physical factors such as recharge etc (Green et al., 2011).

REFERENCES


Variations in travel time for N-loading to groundwaters in four case-studies in Ireland: Implications for policy makers and regulators. Irish J. of Agri-Environ Res. 7.129-142.


