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Mountain dairy wastewater treatment with the use of a 'irregularly shaped' constructed wetland (Aosta Valley, Italy)

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Abstract: In mountain areas, economical activities related to milk processing represent both a key source of income and job opportunities. One of the main characteristics of cheese production is the seasonal variability in the volume of milk processed and wastewater production that tend to limit the capacity of ecosystems to absorb their inputs. In alpine environment, the scarcity of plain surfaces and the climatic conditions results in the need for high CW performances of variable nutrient inputs in different seasons. By evaluating a CW seasonal efficiency for dairy wastewaters in a mountain region (Aosta Valley-NW Italy), this research was aimed to understand how performances of nutrient removal could be affected by seasonal shift in temperature and loadings. Results indicate that the “irregularly shaped” CW, designed to fit the natural landscape, shows best organic removal efficiency in winter (93 and 96% mass removal for BOD_5 in summer and winter respectively), in presence of high organic loadings and low temperatures. Even if nitrate removal is more variable during seasons (71 and 33% mass removal in summer and winter respectively) and differently affected by environmental conditions, overall performance meet the need of high removal efficiency.

Introduction

The difficulties of agriculture in mountain areas are conditioned by their intrinsic features: restricted accessibility, fragility, marginality, steep slopes, altitudinal affected temperatures, and specific niches. These specificities generate circumstances that present at the same time limitations and opportunities (Jodha, 2000). For instance, in Vallée d'Aoste Region (VdA) in NW Italy one of these specificities is the production of cheese (Bassanino et al., 2011). Just as a term of comparison in Europe, EU28, the average annual production of cheese is 1 kg per citizen (source Eurostat) while VdA produces over 30 kg (source Fontina Protected Designation of Origin Cheese Association). This confirms that mountainous European areas are the traditional crib of most typical cheeses: pasture and cheese are in fact both linked to the survival of many familiar activities, being a key source of income. Nevertheless, in mountain areas one of the most significant problems is that of milk processing. In the late 90's, the EU issued guidelines for the production of cheese in the mountains in order to: (i) protect consumers by ensuring hygienically perfect products; (ii) improve the level of hygiene of the structures respecting the tradition (iii) safeguard traditional products. In this sense, the guidelines established the minimum structural requirements of local processing and maturation for which they have been granted derogations for plants of small capacity and sewage processing. In order to ensure quality standards and cost effectiveness cheeses are collectively produced in lowland areas where the main problem is the waste management due to the intrinsic seasonal variability in the volume of milk processed and of wastewater produced (Penati et al., 2011). In fact, during summer, the animals remain at high altitudes and the cheese production in the valley is limited. Conversely in winters everything is concentrated in the low valley. In particular, the production of wastewaters, from both livestock and dairy products under mountain conditions typically tend to limit the capacity of ecosystems to absorb their inputs. The situation is complicated by the fact that the elements of landscape also should be used to valorise Protected Designation of Origin special cheeses in their area of production (Vollet et al., 2008).

A solution to treat wastewater produced by cheesemaking factories could come from constructed wetlands (CWs). CWs systems are designed and constructed to utilize the natural processes involving plants, filling materials and microbial communities to treat wastewater. CWs filling substrata support the growth of diverse microbial guilds which contribute as catalysts for the removal of organic and inorganic wastewater components (Faulwetter et al., 2009). Therefore, microbial abundance strongly influences treatment performance but may be heavily affected by operating conditions (Akratos et al., 2007).

CWs are largely used in areas with mild winters, but there is limited understanding about the control of seasonal pattern under cold winter conditions (Albuzio et al., 2009; Comino et al., 2011; Foladori et al., 2012; Kowalik et al., 1998; Sharma et al., 2013; Züst and Schönborn, 2003). Moreover, most literature about CWs is related to systems designed on larger dedicated surfaces, with defined and regular shapes, without any integration with the pre-existing landscape.

This paper is concerned with the seasonal performance of a CW for the treatment of wastewaters from a medium-size cheese-making factory, designed to fit on the existing landscape. The objective was to evaluate the performances of wastewater purification with particular reference to the nitrogen forms and BOD_5 removal.

Materials and methods

The sub-surface flow constructed wetland (SSF-CW) is located in NW Italy at 500 m a.s.l., where a mean annual air temperature (MAT) of 12 ℃ and mean annual total precipitation (MAP) of 430 mm characterise the local climate. It has been built in the summer 2000 to treat the wastewater from a medium-size dairy factory (*Fromagerie de Champagne)*, and its design criteria have been adapted to create a system compatible with the landscape, established in a public garden adjacent to the factory (Figure 1). This SSF CW was designed to fit the natural landscape ; high performance filling substrata were chosen to overcome the scarcity of plain surface and the problems related to cold climatic conditions. The SSF-CW, has been previously studied by Gorra et al. (2007) for the diversity and stability of ammonia-oxidizing communities. It is irregularly shaped and starts with a narrow ditch of 1 m in depth that continues following the topography of the terrain occupying a total surface of 200 m^2. The bottom of the ditch is covered with a plastic film to prevent wastewater from leaching out of the system. The CW is divided into five sections, each one filled with a different material: 1) gravel from metamorphosed limestone, 2) ground ceramic wastes, 3) by-products from magnetite extraction, 4) zeolitite and 5) A horizon of a Dystrict Endoskeletic Cambisol (WRB, 2007) sampled ten meters on the East of the CW, under a permanent meadow. As one of the key issues associated with SSF-CW is its self-clogging (Pozo-Morales et al., 2013), a bed in the first sector was filled with sharp stones of decreasing size. The SSF-CW had been originally planted with *Pragmites australis* (Cav.) Trin. ex Steud (Table 1, Figure 2); Within sectors 3 and 4 reeds were originally intercalated with *Typha latifolia* L. and *Scirpus lacustris* L., and after three years they were completely substituted by the predominant *Phragmites*.

The treatment system starts physically where wastewaters are collected in a settling tank within the plant. From there they are pumped into the SSF-CW where the five sections are connected to each other by a system of pipes. The natural slope of approximately 3–5% facilitates the flow of the wastewater from the tank through the sections by a horizontal sub-surface flow. After the treatment the effluent, depending on treatment efficiency, waters can be used directly in agriculture or pumped again to the wastewater tank. The wastewater enters the CW at intervals with daily volumes of 9–13 m^3. Two flow-meters monitor hourly the influent and effluent from the system. The daily mean temperature of the substrata is monitored by data loggers UTL-1 (Geotest, Zollikofen CH) buried at 10 cm depth in two sections of the CW (section 3 and 5, Figure 2).

Wastewaters entering the system and the final effluents were sampled twice -monthly for a 3-year period and analysed for pH (pHmeter Crison Instruments), Biochemical Oxygen Demand (BOD_5) (APHA, 1992), Total Kjeldahl Nitrogen (TKN) (APHA, 1992). Ammonium nitrogen ($N\text{-}NH_4^+$) was measured colorimetrically (Crooke and Simpson, 1971) and nitrate N ($N\text{-}NO_3^-$) was determined by ionic chromatography (Dionex DX 50, 2 mm system,18 AS9 analytical column with AG9 guard column, 2000). Organic Nitrogen was calculated as difference between TKN and the sum of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$.

Total coliforms and *E. coli* were monitored as Colony Forming Units by counting colonies formed after incubation of serial dilutions of influent and effluent in Petrifilm® count plates (3M Minneapolis, MN, USA).

Total heterotrophic, ammonia-oxidizing, nitrite-oxidizing and denitrifying bacteria measured in CW filling substrata in replicates (3 for each section) collected periodically during the sampling periods. Total bacteria counts in filling materials and wastewater were measured by incubating serial dilution in petri-dishes with Plate Count Agar medium. Ammonia-oxidising, nitrite-oxidising and denitrifying bacteria were measured by MPN technique (Trolldenier, 1997 a, b).

Removal rates (g m^{-2} d^{-1}) for nitrogen forms and BOD_5 were calculated by taking into account the influent and effluent concentrations, the average daily flow rates and the total superficial area of the CW. Removal efficiencies were calculated as absolute mass or volumetric values to avoid misestimating treatment performance efficiencies as a consequence of precipitation and evapotranspiration.

Daily removal rates and efficiencies for BOD_5, total nitrogen and nitrate in different sampling periods were subjected one-way ANOVA tests and significative differences among periods were evaluated by Duncan's post hoc test. For microbial quantifications in CW substrata total season averages were compared. Positive or negative associations between parameters were evaluated by linear correlation analysis *(*Pearsons). Analyses were considered significant when $p < 0.05^{*}$, 0.01^{**}, 0.001^{***}. All statistical analyses were performed using SPSS software (SPSS Inc., Chicago, IL USA*)*.

Results

The system had been allowed to conditioning for two years, from 2000 to 2002. Maeanwhile, just few parameters were measured periodically in order to evaluate the state of the system. These results refer to the period from summer 2003 to spring 2005 when effluent and influent samples started to be collected twice monthly and all performance parameters were measured.

Operational parameters

During the monitoring period the mean daily air temperature ranged between -10 °C in January and +27 °C in July (Figure 3). The temperat ure of the substrata (October 2004 – September 2005) was positively correlated with the air temperature (r= 0.872**). Only during winter the presence of a snow cover of more than 30 cm partially decoupled the temperature of the substrata from the air temperature, resulting in values close to 0 °C (r= 0.268) (Figure 4).

In Table 1 are presented general operational traits of the CW system. Our CW plant treated more than 3000 cubic meters of wastewater per year (annual average is 10 ± 2 m^3 d^{-1}). Daily inputs were rather variable as a function of the production of the factory, with a maximum during spring of 12 ± 1 m^3 d^{-1} and a minimum of 8 ± 2 m^3 d^{-1} during summer (Figure 5). This was due to the fact that in the valleys along the edge of the Alps cattle production is associated intrinsically to transhumance that implies movement between valleys to higher pastures. During summer livestock are fed at high altitude and the production of the cheese occurs directly there, as well as the wastewater connected with the cheese making. In the valley therefore the system of wastewater treatment must be elastic enough to treat from a minimum of 5 and a maximum of 16 m^3 d^{-1}. Thus, although this wetland was designed for a treatment capacity of about 6 m^3 d^{-1}, the real capacity occasionally was 14 m^3 d^{-1}. Different volumes of influent treated have influenced the retention time, which for such reason varied from 15 to 5 days (average value about 7 days).

The percentage reduction in volume at the CW output (Figure 5) exceeds 50% in autumn with minima of 30% during springs. During summer, volume reduction occasionally reached 90%. Volumes of effluent from the CW system were significantly dependent on volumes entering the system ($r = 0.475^{***}$ - data not shown).

Removal performances

The seasonal characterization of the wastewaters entering and outflowing from the CW system is presented in Table 2. Fluctuations in the influent concentrations reflected the seasonal qualitative variations of the wastewater received: the dairy processing was particularly intense during autumn and winter. This variability is in accordance with reported in literature for the agricultural wastewater treated by constructed wetlands (e.g. Vymazal, 2009).

Differences between influent and effluent mass (g) characteristics over all the different seasons (Table 2), result significant ($p<0{,}005$) for all the parameters. Inflow BOD_5 concentration was higher in winter and lower in summer as expected. The majority of total nitrogen inflow (>85%) was in organic form, whereas the concentration of ammonium and nitrate N was lower, 8-14% and 2-4% in respect to total nitrogen respectively. The mean removal efficiency for BOD_5 was higher than 90%, while for the nitrogen ranges between

50-60%, approximately. BOD_5 removal efficiency values were more stable within the seasons. Considering the mass removal in the different seasons, removal efficiencies were higher for BOD_5 respect to other parameters, with an average of 96% in winter and 94% in autumn and lower efficiencies in spring and summer, 93 and 81% respectively. Organic nitrogen decrease was relatively homogeneous in the various seasons, with a higher removal, from 62 to 55%, in autumn and winter and a lower removal in spring and summer, ranging between 55 and 45%. Despite ammonium nitrogen (N-NH_4^+) and nitrate N (N-NO_3^-) were less abundant in wastewater entering the system, the removal of inorganic N presented a lower efficiency, ranging between 47 and 70% for N-NH_4^+ and 33 and 77% for N-NO_3^-, and different seasonal trends compared to BOD_5 removal, with the higher removal efficiencies detected in the summer season.

Influent showed a rather variable pH of 5.5 in average, with minima during winters and maxima in late summers/autumns. The passage through the CW sections increased its pH and decreased its seasonal variability; the output water has an average pH value of 7 (Figure 6). All of the metal species present in solution possibly precipitate during the flow through the CW. In the case of nitrogen, the equilibrium is shifted from ammonia to nitrate forms. In the case of carbon, the acetate which is one of the most common intermediate of anaerobic respiration (which certainly occurs in some area of some sector of CW), at pH 7 under oxidising conditions shifts the equilibrium toward CO_2, $HCOO^-$, $C_2O_4^{2-}$ (Lindsay, 1979). These reactions facilitate the transformation and eventually a partial removal of the organic matter.

Even if pH of the inflow was low, wastewaters presented a high total bacteria count, 10^8 UFC mL^{-1} (Table 2). Despite these high counts, average total bacteria removal efficiency of CW was about 99%, with 106 UFC mL^{-1} only in the effluent. Total and fecal (*E. coli*) coliforms are typical components of wastewater and strongly influence wastewaters discharge/disposal criteria. They were present in influent most likely because of their usual presence in raw milk but decreased in effluent with a reduction of 95 and 100% for total and fecal coliforms, respectively.

Microrganisms

Total heterotrophic aerobic, denitrifying, autotrophic ammonia-oxidizing and nitrite-bacteria present in filling materials of the CW were quantified in order to evaluate the stability of the microbial groups involved with the treatment performance (Figure 9). Heterotrophic aerobic bacteria presented a stable abundance during the different seasons, with very low standard deviations. Ammonia-oxidizing, nitrite-oxidizing and denitrifying bacteria, more involved in nitrogen removal, presented significant differences over the different seasons, with different trends. Ammonia-oxidizing and nitrite-oxidizing bacteria, autotrophic aerobic groups that oxidize ammonium to nitrate N were more abundant in autumn, whereas denitrifying heterotrophic bacteria, involved in nitrate N reduction and volatilization were more abundant in spring.

Discussion

Traditional constructed wetlands are normally designed on larger dedicated surfaces, with regular geometry, without any integration with the pre-existing landscape. After plant colonization, the irregular aspect of this CW appeared integrated with the existing landscape (Figure 2) looking more a natural than a constructed ecosystem. Despite this, operational traits were comparable to CWs built in the same environmental conditions and in general, our results do not differ from similar wastewaters (Albuzio et al., 2009; Biddlestone *et al.*, 1991; Mantovi et al., 2003; Masi et al., 2007; Merlin et al., 2002; Newman *et al.*, 2000; Comino et al., 2011).

Even considering seasonal loading variability, BOD_5 and hydraulic inputs in the CW system were higher respect to many SSF constructed wetlands systems with larger surfaces, as reported by Vymazal (2009). Despite these high inputs, treatment performances were elevated, especially for BOD_5 removal that reached an average mass removal efficiency of 96% during the winter season. The treatment performance resulted rather stable over an extended period of time and did not show any declining tendency.

Seasonal removal efficiencies of nitrogen forms were lower than BOD_5 but anyway significant: 45-62%, 47-70% 33-77% for organic, ammonium and nitrate N, respectively. Nevertheless these treated wastewater concentration values are ten times lower than the threshold limit for nitrate concentrations in waters in Europe, equal to 50 mg L^{-1} according to both Water Framework (2000) and Groundwater (2006) EU Directives. Some parameter of the effluents like pH, nitrate N, total phosphorus and microbiological indices satisfied the Italian national limits for discharging in superficial waters (pH 5.5-9.5; NO_3^- 20 mg L^{-1}; P_{tot} 10 mg L^{-1}, *E. coli* 5000 ufc mL^{-1}; G.U., 2006). Depending on the seasons, BOD_5 and ammonium effluent concentrations fall within the same discharging class (BOD_5 40 mgL^{-1} O_2; NH_4^+ 15 mg L^{-1}) or, at least, with the limits of the sewer system discharge. The CW was also effective for wastewater treatment and removal of pathogens. Significant linear, positive correlations were observed between incoming mass loads of BOD_5, TN, N-NH_4^+ and N-NO_3^- and the respective removal rates (Figure 7). In particular, BOD_5 removal rates showed very strong association with loadings (r^2 = 0.98***). This trend, already described in literature but mostly related to CW systems in temperate or tropical environments, indicate that, in general, subsurface CWs systems may exhibit higher treatment performance at higher loadings (Trang et al., 2010), independent on the climatic conditions, which in this area are characterized by low precipitation and sharp differences between cold winters and warm summers (Mercalli, 2003). At the same time, a variable wastewater supply and different seasonal retention times influenced the proportion of the different components in the effluent, possibly due to variations on the contact time between wastewater and microorganisms.

Different ability to remove organic or inorganic molecules are shown by the different relative seasonal concentrations of organic, ammonium and nitrate N in the influent respect to the effluent and by the comparison of the trends in BOD_5 vs N-NO_3^- removal, rates of which were influenced in an opposite ways by organic loadings (Figure 8). Moreover, although the same hierarchic order was maintained (most of the nitrogen in organic form), the relative percentage of inorganic nitrogen was higher in the effluent with respect to the influent. The fact that the inorganic nitrogen removal is a critical step in the range of the total treatment performance of the system is demonstrated by the correlation of N-NO_3^- removal rate with some functioning-environmental parameters (Table 3), while the other removal rates were not associated to the same parameters. In particular, the correlation between N-NO_3^- removal and air temperature indicates that lower temperatures negatively affected N-NO_3^- removal efficiency, whereas best performances of organic carbon mineralization occurred in autumn and winter seasons when the hydraulic loading was higher and nutrients in wastewater were also more concentrated.

In CWs, the real effect of temperature on the depuration process depends on various operating characteristics that can protect the depuration efficiency from harsh environmental conditions (Kadlec and Knight, 1996). Huang et al. (2013) suggested that appropriate heat-preservation measures of covering the surface of SSF-CWs could preserve microbial activity during cold seasons. In the CW under study, during winter, the presence of a consistent snow cover maintained the temperature of the substrata close to 0 °C, independent on the air temperature. It has been demonstrated that soil protected in this way can remain in a partially unfrozen condition (+1 to −3 °C) (Freppaz et al., 2008) and that biological activity is surprisingly high at temperatures approaching subzero, which are typical of these subnivian conditions (Freppaz et al., 2007a, b).

Organic carbon mineralization and ammonification result from a complex of heterotrophic aerobic microbial activities occurring in a wide range of environmental conditions, and are therefore less influenced by environmental variations. These bacteria, which have a relatively fast metabolic rate, were always abundant and stable in this CW system during the different seasons. During the winter, when the organic input was high, as demonstrated by high BOD_5 removal performances, they were active in mineralization, producing both CO_2 and $N\text{-}NH_4^+$. The $N\text{-}NH_4^+$ produced with the inorganic nitrogen already present in the influent are removed by ammonia-oxidation and, subsequently, by denitrification. These last microbial processes require alternating oxic-anoxic conditions (Brix, 2003) and are carried out by slower-growing bacteria, which were less abundant and more variable among seasons (Figure 9). These evidences are corroborated by the findings that, beside the relative genetic stability of the microbial community within the system, the potential activity in the system were significantly different in the different seasons. This was demonstrated by Gorra et al. (2007) on the same CW system.

Nitrification and denitrification, are more dependent on environmental and operating conditions (Saeed et al., 2012), they are in many cases the limiting processes for total nitrogen removal (Chang et al., 2012) and CWs design criteria often have the purpose to optimize the nitrification-denitrification pathway (Pan et al., 2013, Ding et al., 2012).

Therefore, thinking about changes in order to optimize the inorganic nitrogen removal facing a possible increase in CW loading, it could be necessary, in a further design phase, to enhance the nitrification process, for example by increasing the residence time (or water saturation) in the last section of the CW.

Conclusions

The results obtained in the wetland under study, showed that the functional characteristics of its construction allow consistent depuration efficiency, in particular as far as the organic matter mineralization from wastewaters is concerned. The design and the operability of this irregularly shaped SSF-CW, designed to fit on the existing landscape, were positive and with low costs, not sensitive to peak flows, even if compared to similar plants in the same environment. Moreover, filling substrata and operational characteristics of the CW seems to efficiently and constantly support the aerobic microbial mineralization, in particular in winter seasons when cheese-making activities are more concentrated.

The design of the CW has avoided the self-clogging and final effluent has a final quality that would, at least, permit their use in agriculture. Having assessed that this CW generally worked better for the organic matter mineralization when it is fed more, at least in the range of loading considered, it is necessary to take into account that inorganic nitrogen removal (in particular the nitrate), could be more affected, with respect to other removal rates, by a heavy input of organic matter and by seasonal variations in microbial populations. Therefore, thinking about changes in order to optimize the inorganic nitrogen removal facing a possible increase in CW loading, it could be necessary, in a further design phase, to enhance the denitrification process, for example by increasing the residence time (or water saturation) in the last section of the CW.

This study demonstrates how a non-standard CW, designed with an irregular shape, fitting the natural landscape, may present a high performance respect to traditional CW. However it should be pointed out that CW treatment efficiency may be affected by changes in nutrient loadings, which are possible considering that agricultural wastewater are intrinsically variable in composition and irregularly produced. For this reason it is necessary to consider the possibility of modulating operational aspects, as for example the HRT, in order to build ‘flexible’ systems that could maintain high performance even in presence of significant changes of production processes.

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Table 1. Sub-surface flow-constructed wetland (SSF-CW) by the *Fromagerie de Champagne*.

		Champagne 45°44'30.86"N 7°31'32.92"E
	m a.s.l.	534
Pre-treatment		ST[#]
Pump station	kW	no
Flow system		SSF[†]-V1 SSF[†]-V2 SSF[†]-V3 SSF[†]-V4 SSF[†]-V5 tank
Tank	m^3	45
Total area	m^2	500
Pool (total operative surface)	m^2	200
Inflow	m^3 d^{-1}	9
Load (BOD_5)	mg L^{-1}	≈800
HRT[††]	d	8
HLR[‡‡]	m^3 m^{-2} d^{-1}	44
Vegetation		(TYL, SCL), PHA[*]
Investment (total costs)	EUR m^{-2}	67[¶]

[†] SSF sub-surface flow

[#] ST settling tank

* PHA *Phragmites australis* (Cav.) Trin. ex Steud., TYL *Typha latifolia* L., SCL *Scirpus lacustris* L.

¶ inflation adjustment, cost actualised at year 2013

††HRT (Hydraulic Retention Time) calculated according to Kowalik et al. (1998) where V = capillary capacity equal to active volume in m^3 and Q = mean average flow rate per day of wastewater through the system m^3d^{-1}). For the volumes calculation an average 20% porosity was assumed.

‡‡HLR (Hydraulic Loading Rate) calculated according to the Kadlec and Knight (1996) equation

Table 2. Average seasonal CW performances during the period 2003-2005.

		Concentration		Mass		Removal %	
	Season[†]	In[‡]	Out[‡]	In[‡](g)	Out[‡](g)	Conc[#]	Mass
pH	Wi	4.9	7.1				
	Sp	4.5	6.8				
	Su	5.3	7.1				
	Au	5.9	7.4				
BOD_5* $/mg\ O_2\ L^{-1}$	Wi	925	71	9208	380	92	96
	Sp	858	249	9883	1910	71	81
	Su	480	59	3622	257	88	93
	Au	906	89	9363	547	90	94
TN $/mg\ L^{-1}$	Wi	147	107	1421	624	27	56
	Sp	186	123	2160	961	34	56
	Su	248	242	2070	1089	2	47
	Au	211	151	2307	886	28	62
N org $/mg\ L^{-1}$	Wi	129	92	1245	543	29	56
	Sp	162	107	1883	833	34	56
	Su	227	230	1912	1040	-1	46
	Au	180	131	1978	763	27	61
$N\text{-}NH_4^+$ $/mg\ L^{-1}$	Wi	18	15	176	81	17	54
	Sp	22	17	256	134	23	48
	Su	20	12	158	48	40	70
	Au	30	20	330	123	33	63
#$N\text{-}NO_3^-$ $/mg\ L^{-1}$	Wi	3	4	27	18	-33	33
	Sp	5	5	59	35	0	41
	Su	8	3	62	14	63	77
	Au	10	5	105	27	50	74
P tot $/mg\ L^{-1}$		10	6	72	30	40	58
Bacteria tot $/UFC\ mL^{-1}$		7.12×10^8	3.14×10^6			99.6	
E.coli $/UFC\ mL^{-1}$		3.15×10^2	0			100	
Coliforms tot $/UFC\ mL^{-1}$		1.52×10^3	6.67×10^1			95.6	

[†]Winter (Wi) Dec-Feb, Spring (Sp) Mar-May, Summer (Su) Jun-Aug, Autumn (Au) Sep-

Nov

‡Seasonal averaged values. Standard errors always <15%

#Concentration

*Decreto Legislativo 3 aprile 2006, n. 152 "Norme in materia ambientale" Gazzetta Ufficiale n. 88 del 14 aprile 2006 – Suppl. Ord. 96

91/676/CEE, 2006/118/CE, 98/83/CE N-NO_3^- 50 mg L^{-1}.

Table 3. Correlations between N-NO_3^- removal and functioning parameters. Where removal efficiencies were calculated as [mass (g) inlet – mass outlet]/mass inlet and removal rates as [inlet – outlet] in grams m^{-2}.

Correlation	Pearson	*p*
† RE N-NO_3^- *vs* N-NH_4^+	0.614	0.015
†RE N-NO_3^- *vs* T	0.697	0.004
‡ RR N-NO_3^- *vs* HLR	0.504	0.017

†RE Removal Efficiency

‡RR Removal Rate

Figure 1. Sub-surface flow-constructed wetland (SSF-CW) by the Fromagerie de Champagne that produces Fontina cheese and butter. Fontina Vallée d'Aoste is made from unpasteurised milk from a single milking, with two batches per day. In the whole region the Fontina producers are around 400 to market 3,500 tons of cheese per year. The "*Champagne Società Cooperativa*" processes about 8 ± 4 m^3 of milk produced by small less than hundred farms. A settling tank is located at the bottom of the factory by the CW inlet pipe; the entire flux within the SSF-CW sectors occurs by gravity. The outlet pipe runs into the tank.

Figure 2. Details of the sub-surface flow constructed wetland. On top left, map of sections (Gorra et al., 2007): 1) indicates the filter section composed by a series of gravel sectors with size classes from pebble to granular, 2) indicates grounded ceramic wastes section, 3) indicates magnetite mine wastes section, 4) indicates pellets of zeolitite minerals section, 5) indicates the section filled by unconsolidated rock fragments with a decreasing particle size range that includes size classes from granule- to boulder-sized fragments. Dots indicate sampling point for the solid phase analyses [courtesy of The Society of Applied Microbiology]. Pictures from top right indicate: the stages of construction (1a), (1b), and (1c) in September 2000; Eastern view during summers of 2003(2), 2004(3), and 2005 (4); Southern view in December 2012 (5).

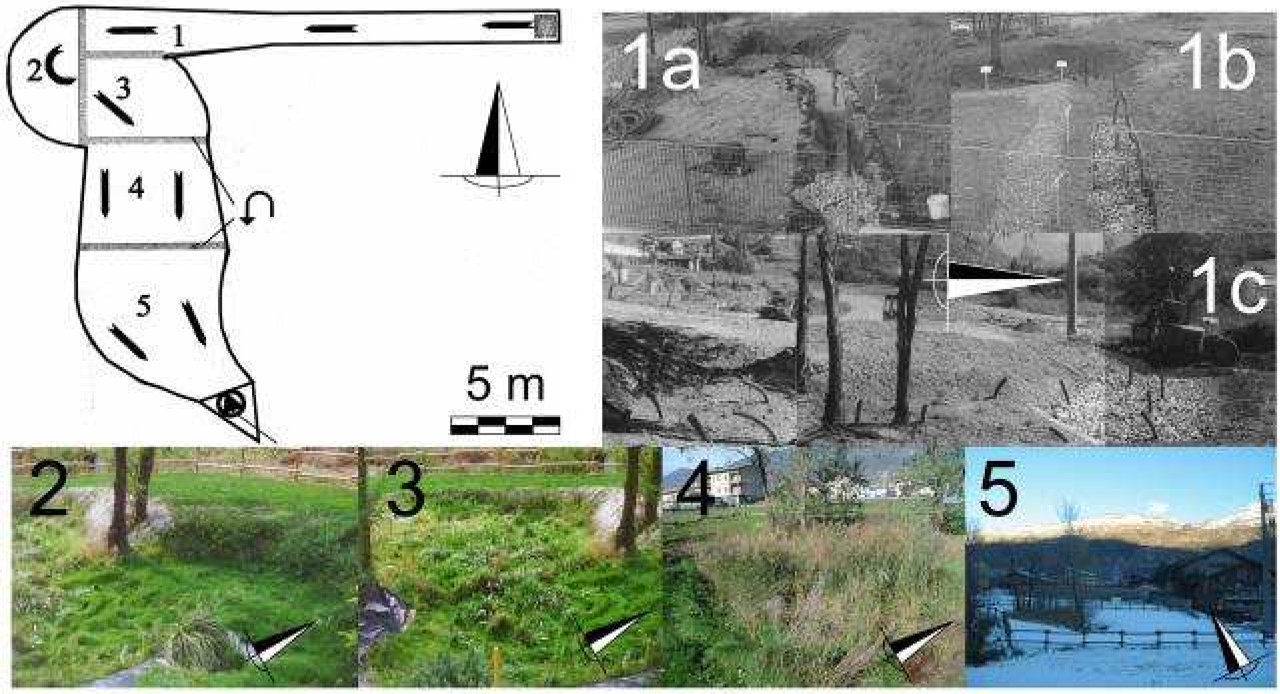


Figure 3. Three monthly cumulative precipitations (above) and mean daily air temperature (below): Saint Christophe (■, 45°44’N7°22’E) and Villeneuve (×, 45°42’N7°12’E) are the closer stations to Champagne, while Morgex (O, 45°45’N7°02’E) is shown for comparison.

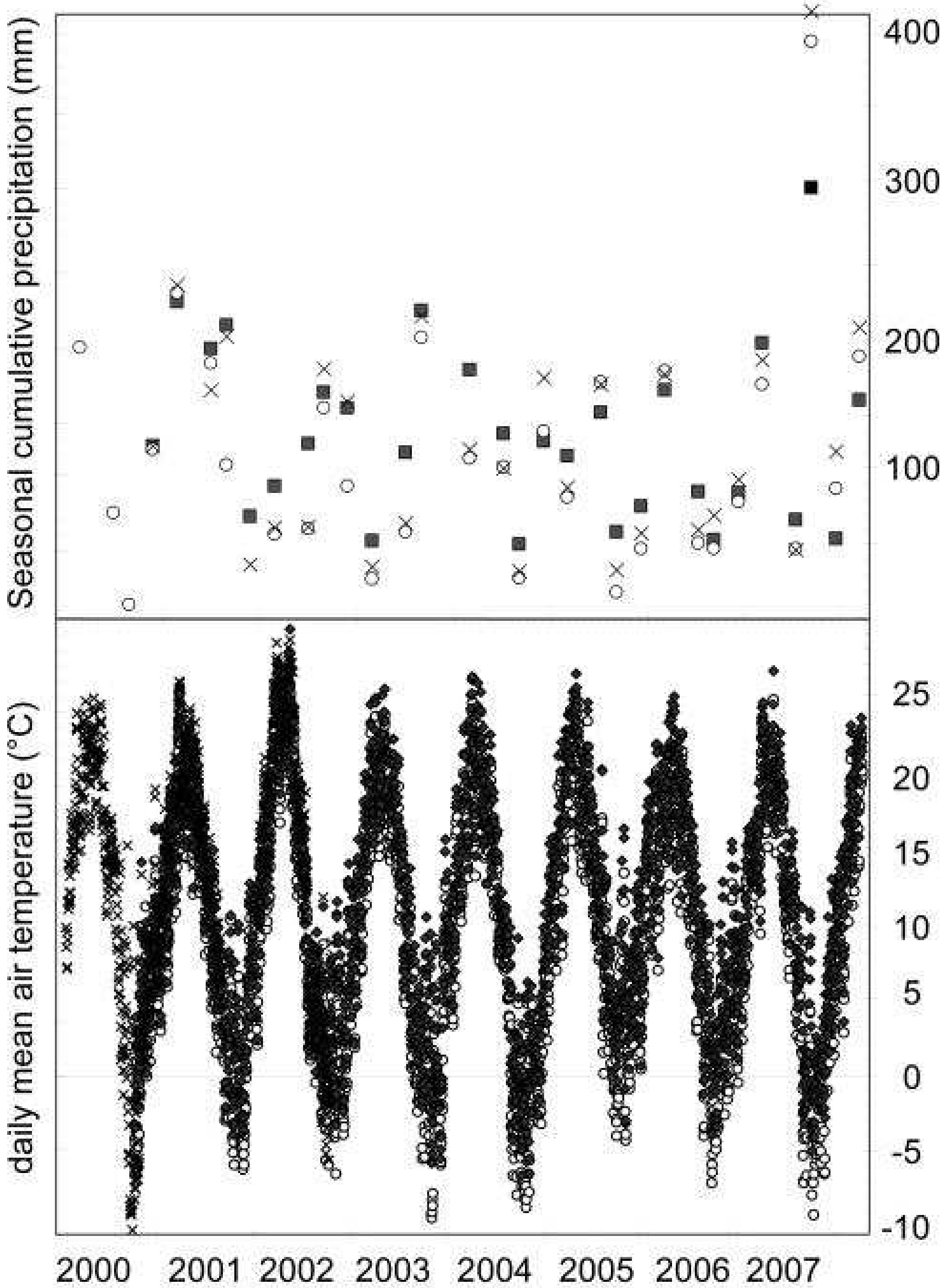
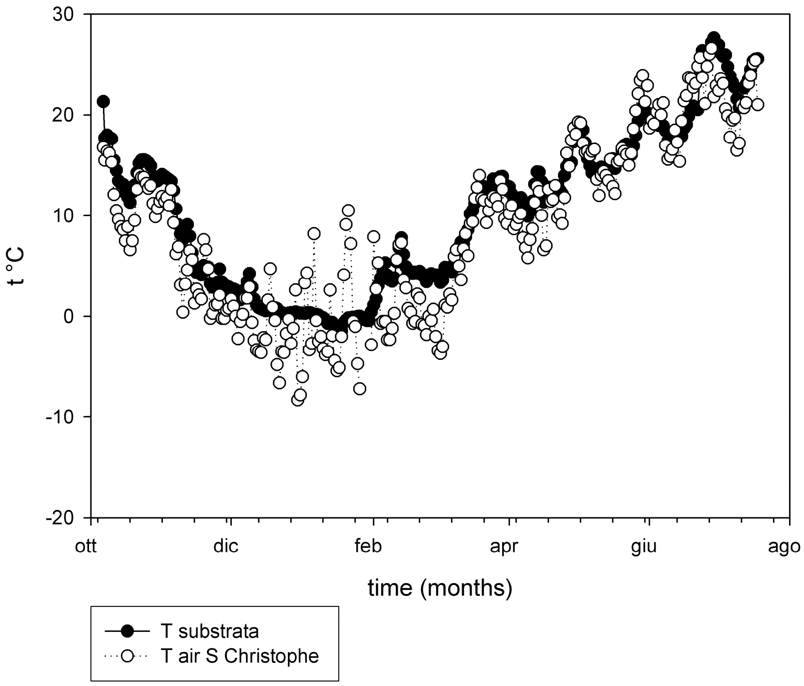


Figure 4. (a) Open circles indicate daily measurements of air temperature (Saint Christophe, 45°44'N 7°22'E), while closed circles t he temperature of the substrate at 10 cm depth (year 2004-2005). **(b)** Temperature of substrata against air temperature. Units are Celsius degrees.



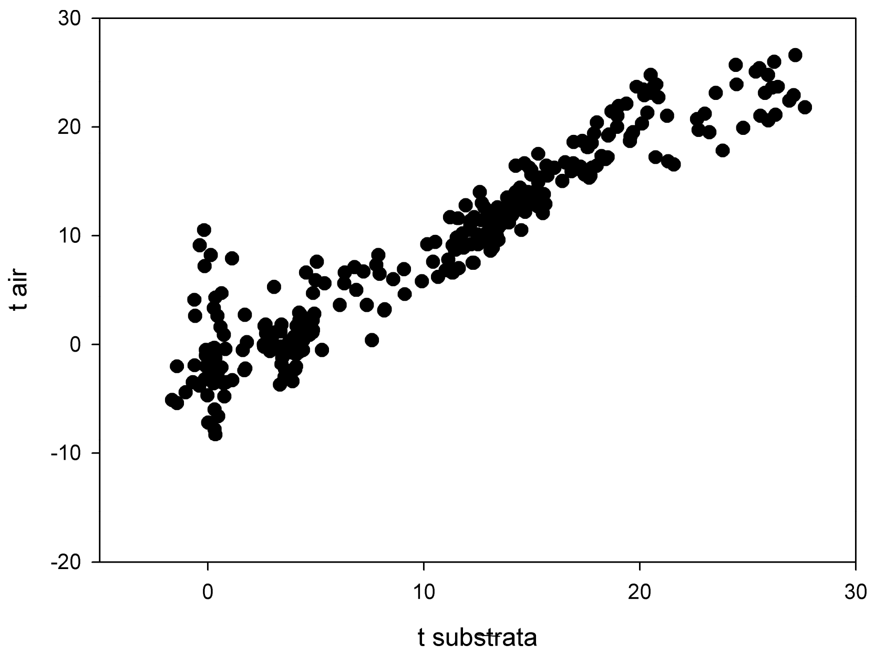


Figure 5. Daily cubic meters entering (filled histograms) and outflow (open histograms) from the systems over each individual season [Autumn (Au) (Sep-Nov), Winter (Wi) (Dec-Feb), Spring (Sp) (Mar-May), Summer (Su) (Jun-Aug)]. Standard deviations over the whole observed period are shown.

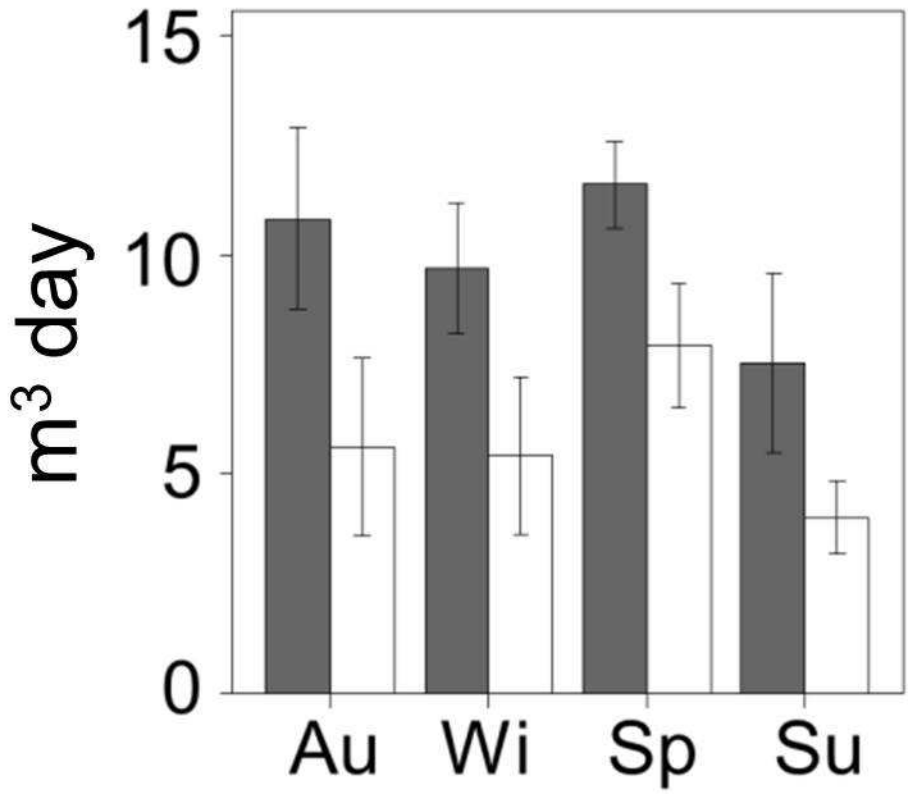


Figure 6. Monthly measurements of pH of the wastewaters (open circles) and the output from the constructed wetland (closed squares). Individual sections from 1 to 5 are represented by pale grey to black diamonds.

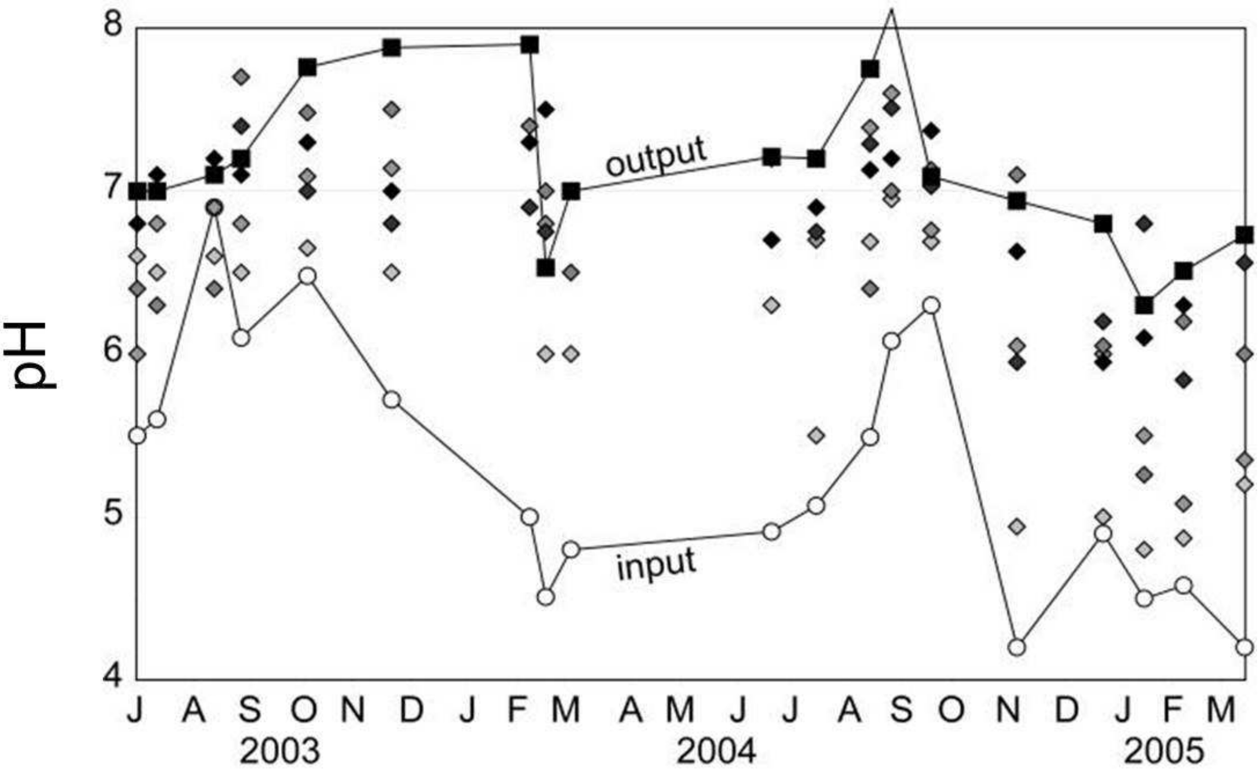


Figure 7. Loadings and removal rates BOD_5**, Total N**, NH_4^+**,NO_3^-**. Unit g m^{-2} day^{-1}. Symbols indicate: (◇) December-February, (□) June-August, (△) March-May,(○) September-November.

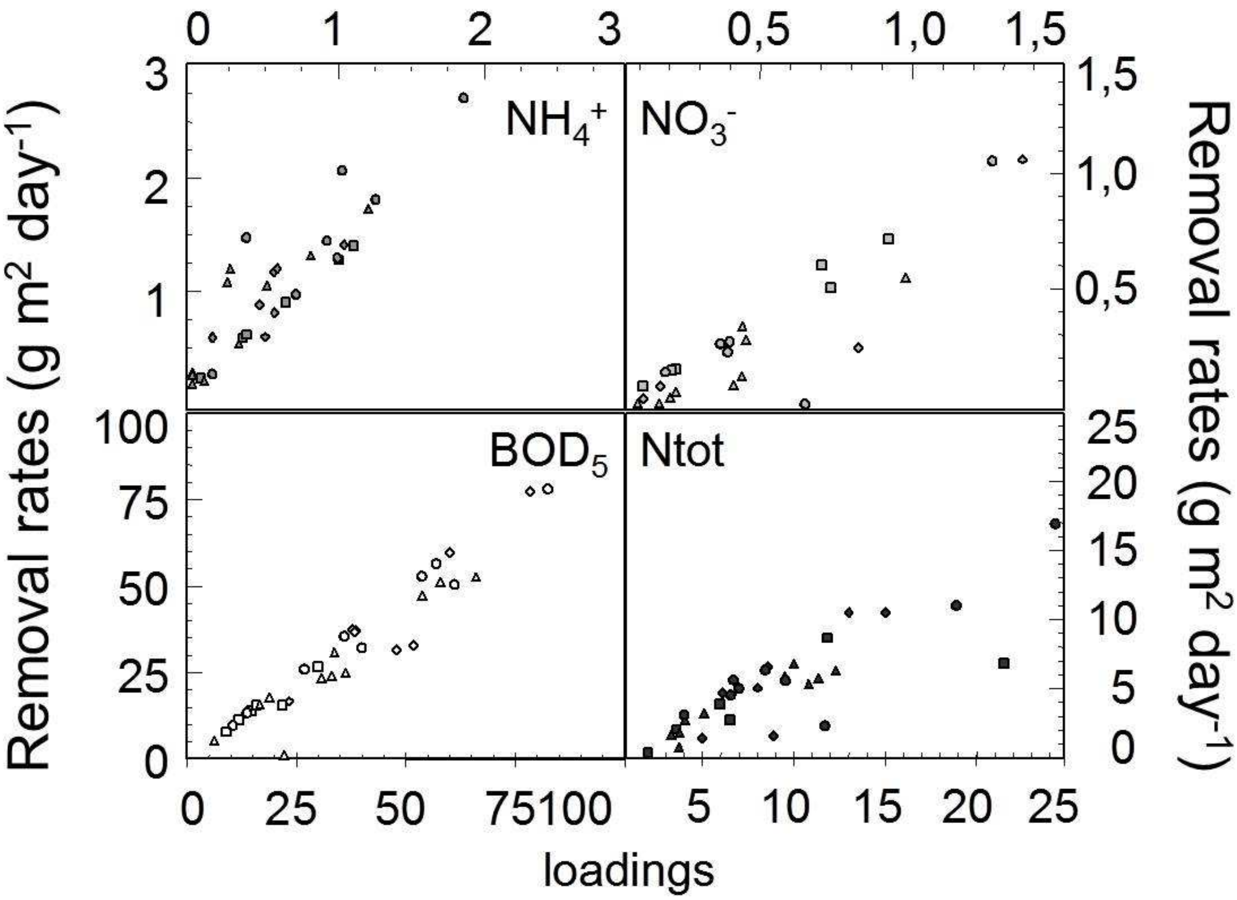


Figure 8. N-NO_3^- (above) and BOD_5 (below) removal rates at different loading rates conditions in different seasons of CW functioning (histograms). Open circles indicate organic loading rate. Units are g m^{-2} day^{-1}.

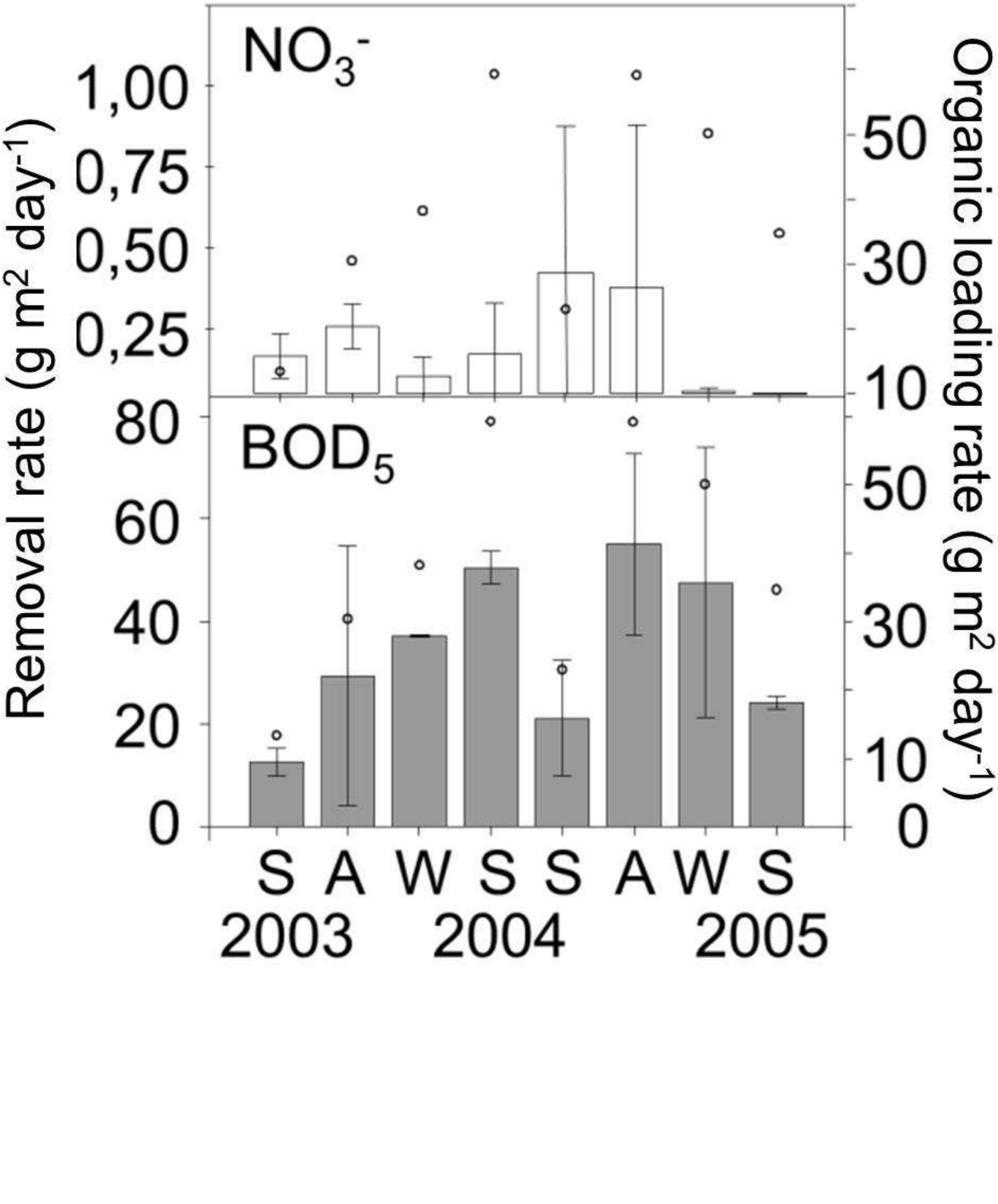


Figure 9. Microbial counts of total heterotrophic bacteria (black histograms), ammonia-oxidizing bacteria (dark grey histograms), nitrite-oxidizing bacteria (white histograms) and denitrifying bacteria (light grey histograms) in the filling material of the CW. Standard deviations are shown. Different characters indicate significant differences (p<0.05) among seasonal means according to Duncan's *post hoc* test. Regular, italic and capital font are referred to ammonia-oxidizing, nitrite-oxidizing and denitrifying data sets respectively.