

## Microbial Eco-Physiology of Degrading Aral Sea Wetlands: Consequences for C-Cycling

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**The effects of elevated salinity level on the physiology of microbial communities and C-cycling were evaluated in soils located in areas near the degraded Aral Sea. One site on a freshwater wetland (FW) and two sites on a salt water wetland on the eastern (EW) and northern shores (NW) with salt contents of 1.1, 34.3, and 78.9 g L<sup>-1</sup>, respectively, were selected. The total microbial biomass estimated by fumigation-extraction and the respiration rate were lower at the two sites with elevated salinity level (EW and NW) than in FW while the amount of extractable organic carbon (EOC) was higher. The EOC/biomass-C ratio as an index of carbon supply was highest in NW where the lowest microbial activity was detected, indicating the restriction of microbial metabolism. Thus elevated salinity level appeared to restrict microbial colonization of organic substrates and C transformation rate but to increase the amount of soil available C. Difference in precipitation (rain events) between 2002 and 2003 did not affect the respiration rate and surface CO<sub>2</sub> flux in FW. In NW, the respiration rate increased by more than 5 times, suggesting that in the ecosystems with a high content of available C, elevated CO<sub>2</sub> emissions may occur under changing environmental conditions.**

**Key Words:** land degradation, microbial eco-physiology, organic matter, salinization, wetland.

Microbial response to rising salinity has attracted a great deal of interest because of its strong effect on ecosystem processes and biodiversity (Esechie et al. 1998; Leland and Fend 1998). The effects of changes in the salinity level on rate nutrient transformation have been reported (Badia and Alcaniz 1993; Sarig and Steinberger 1994; Inubushi et al. 1998). Groffman et al. (1995) observed that nitrification was more sensitive to rising salinity than denitrification. Imbalance between the processes under changing salinity levels induced elevated N<sub>2</sub>O emissions (Inubushi et al. 1999).

Several environmental problems, including the depletion of drinking water resources, reduction of fish diversity and respiratory diseases due to a rising salinity level occur in the Aral Sea region, which is located in the arid zone of Central Asia. Aral Sea was the second largest closed sea on Earth. At the end of 1950, degradation started, resulting in the loss of 80% volume and 50% surface. These drastic changes were caused by the reduction of total influx due to intensive irrigation developed along two feeding rivers. At present the Sea is divided into two lakes and the areas of exposed floor

cover 2.5 million ha. As a result, salt concentration in water drastically increased. Although the influence of the rising salinity level on nutrient cycling has been extensively investigated, the transformation of carbon in highly salinised ecosystems has not been fully elucidated. In the present study, eco-physiological approaches in which microbial communities are considered as a single organism were adopted to evaluate the effects of elevated salinity level on microbial biomass, respiration rate and C-cycling in Aral Sea wetlands.

### MATERIALS AND METHODS

**Soils.** Three wetland ecosystems with different salinity levels were selected in the study as follows: a freshwater wetland (FW), a wetland located on the eastern shore (EW) and on the northern (NW) shore of the Aral Sea. Table 1 shows the physicochemical characteristics of the selected soils. The soil from the FW ecosystem was classified into freshwater sandy flood-lands and the soils from EW and NW into alkali-marsh Solon-

**Table 1.** Physicochemical properties of the soils used.

	Freshwater wetland (FW)	Eastern wetland (EW)	Northern wetland (NW)
Position	N 46°09'081" E 61°15'286"	N 46°21'829" E 61°13'676"	N 46°36'840" E 60°46'802"
Total C (mg g <sup>-1</sup> soil)	18.2	30.4	21.3
Total N (mg g <sup>-1</sup> soil)	1.67	1.20	1.27
Salinity (g L <sup>-1</sup> )	1.1	34.3	78.9
Bulk density (g cm <sup>-3</sup> )	1.40	1.43	1.64
pH(H <sub>2</sub> O)	8.25	9.20	9.98

chaks. Three independent samples, consisting each of approximately 20 sub-samples, were collected from the representative ecosystems in 2002 and 2003. The soil samples were kept at 4°C and preincubated under natural water-saturated conditions in an anaerobic N<sub>2</sub>-atmosphere at 25°C for 10 d before the analyses were conducted.

**Analyses.** Total microbial biomass, respiration rate and amount of extractable organic carbon (EOC) were monitored. The amount of microbial biomass C was measured by the fumigation-extraction technique (Vance et al. 1987) using  $k_{\text{EC}} = 2.64$  with some modifications of the method developed by Inubushi et al. (1991) for flooded anaerobic soils. The amount of EOC was determined using a total organic carbon analyzer (TOC-5000, Shimadzu) with a catalyzed dry-digestion procedure. To extract organic carbon, samples of moist soil equivalent to 10 g on an oven-dry basis were shaken together with 40 mL of a 0.5 M K<sub>2</sub>SO<sub>4</sub> solution for 0.5 h using an end-to-end shaker and filtered through Whatman paper filter No. 625. Respiration rate of the soil microorganisms was calculated based on the CO<sub>2</sub> emission rate during the incubation of fresh soil (2 g of oven-dry material) at 22°C. The amount of CO<sub>2</sub> emitted was determined by gas-chromatography with a thermal conductivity detector. The closed soil cover box technique was applied to monitor the CO<sub>2</sub> flux from soil into the atmosphere under field conditions (Inubushi et al. 1998). Gas sampling was conducted three times for 2 h after closing to calculate the average value. Chemical oxygen demand (COD) and biological oxygen demand (BOD) were additionally determined at 25°C in soil samples equivalent to 2 g on an oven-dry basis (Greenberg et al. 1992).

All the measurements were carried out in triplicate. Standard deviations were calculated for all the data. The difference was considered to be significant at  $p < 0.01$ .

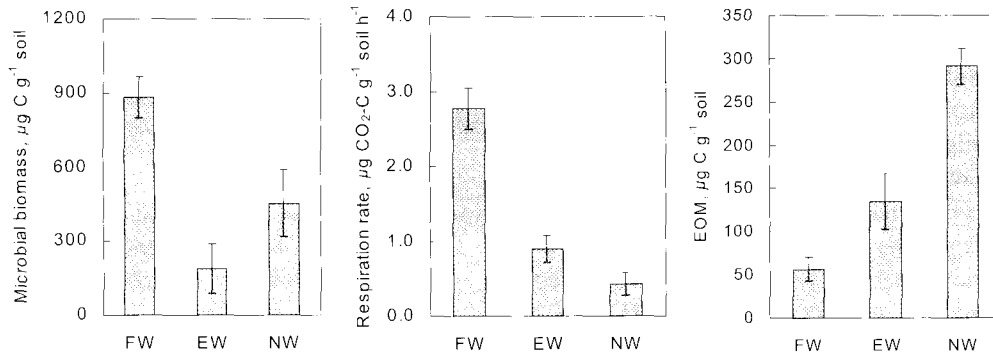
## RESULTS AND DISCUSSION

Although the total microbial biomass in the soil of the salt wetlands was more than two times lower than that in the freshwater ecosystem, the higher salinity level in NW than in EW did not result in a lower microbial bio-

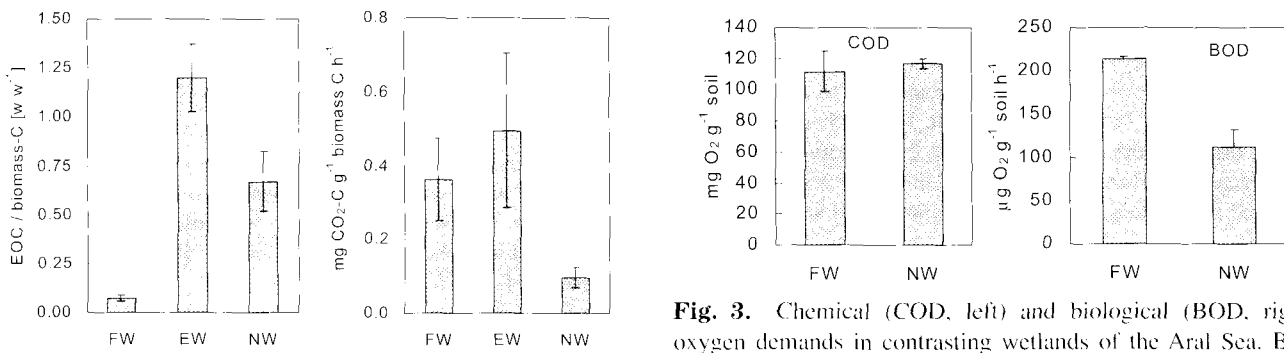
mass (Table 1, Fig. 1). The rate of microbial respiration decreased with the increase of the ecosystem salinity level, suggesting a reduction in the carbon transformation rate. In contrast, the content of EOC was well correlated with the salinity. The availability of most carbon sources depends on their solubility and extractability, as demonstrated by Stucki and Alexander (1987) who studied the degradation of aromatic compounds. Accumulation of available C detected under reduced mineralization rate in EW and NW seems relation to findings by Inubushi et al. (1999) who observed accumulation of NH<sub>4</sub><sup>+</sup> as a result of reduced nitrification at the elevated salinity. In addition, Jones et al. (2004) noted that dissolved organic nitrogen is an important source of N for microorganisms. Thus, the EOC fraction representing the dynamic value apparently reflects the amount of available C pool in soil.

To analyze the effect of an elevated amount of EOC in salt wetlands, we calculated the EOC quotient per biomass unit. The quotient is assumed to indicate the nutritional conditions under which microbial biomass occurs and thus may be considered as an index of carbon supply. Here, the microbial biomass under elevated C-supply in the two saline wetlands (EW and NW) showed a significantly lower respiration rate than that in the freshwater ecosystem (Figs. 1 and 2). Metabolic quotient representing the respiration rate per biomass unit indicated that the portion of physiologically active microorganisms in the total microbial complex did not differ between the FW and EW ecosystems, while the component was significantly suppressed in the highly salinized NW ecosystem. Thus the lowest portion of active microorganisms and lowest respiration rate under the highest C-supply suggest that the metabolic capability of the microbial communities was restricted, which may account for the accumulation of EOC in such ecosystems.

The COD quantitatively reflects the total amount of oxidizable substrates while the BOD characterizes the availability of these substrates for biological oxidation under controlled conditions. COD values were similar in the FW and NW soils (Fig. 3). In contrast, the BOD value in NW was half of that in FW, indicating that a lower biological activity had developed under similar

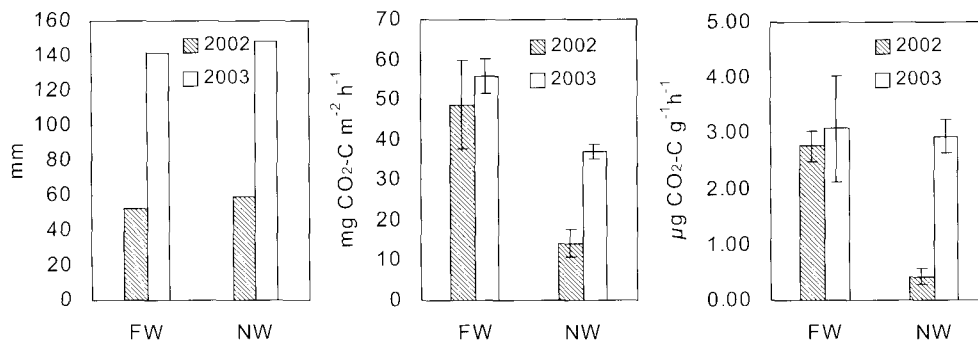


**Fig. 1.** Microbial biomass, respiration rate, and amount of extractable organic carbon (EOC) in three contrasting wetlands of the Aral Sea. FW, freshwater wetland; EW, eastern wetland; NW, northern wetland. Bars represent values of standard deviation.



**Fig. 2.** C-supply index expressed as ratio of extractable organic carbon (EOC) to total microbial biomass (left) and metabolic quotient (right) in three contrasting wetlands of the Aral Sea. Bars represent values of standard deviation. See the legend to Fig. 1 for abbreviations.

**Fig. 3.** Chemical (COD, left) and biological (BOD, right) oxygen demands in contrasting wetlands of the Aral Sea. Bars indicate the value of standard deviations. See the legend to Fig. 1 for abbreviations.



**Fig. 4.** Precipitation during the period of March–July in 2002 and 2003 (left), surface  $\text{CO}_2\text{-C}$  emission (middle) and respiration rate (right) in contrasting wetlands of the Aral Sea. Bars indicate value of standard deviations. See the legend to Fig. 1 for abbreviations.

supply with potentially oxidizable substrates and at a higher EOC content (Fig. 3). Since, generally, the COD and BOD values correspond to one another, a difference between them may indicate how favorable the environmental conditions are for microbial activity. The lower BOD value in NW apparently suggests that when the salinity level is high, a significant portion of substrates becomes unavailable for microbial oxidation. Thus an elevated salinity level restricts the microbial activity and

microbial contribution to C transformation but increases the amount of available C pool in soil.

A significant difference in precipitation (rain events) was observed between 2002 and 2003 (Fig. 4). This climatic variability did not affect the respiration rate in FW but increased it by more than 5 times in the saline ecosystem (NW). Similarly surface emission of  $\text{CO}_2\text{-C}$  was strongly affected in NW. Although the salinity level only slightly decreased in 2003 and remained relatively high

(63.4 g L<sup>-1</sup>), more frequent precipitation, which leads to temporary desalination of the surface soil, may account for the effect. Elevated respiration values obviously suggest that the microbial activity of the Sea wetlands is restricted by the high salinity level, and that the reduction of salt stress results in elevated carbon losses from soil. Comparison of the data obtained in 2002 and 2003 indicates that ecosystems with an elevated content of available C may be associated with the risk of elevated CO<sub>2</sub> emissions under environmental changes. The areas with an accumulation of significant amounts of available C should be identified to predict the changes in the coming decades.

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