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Nitrogen Fixation in Soils and Canals of Rehabilitated Raised–Fields of the Bolivian Altiplano[1]

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ABSTRACT

Raised-field agricultural systems were exploited extensively by pre-Columbian Andean civilizations and now are used less extensively by contemporary Bolivian and Peruvian farmers as a method for farming the perennially wet soils of the intermontane pampas of the Andean high altiplano. The raised-field agricultural systems are linear or semi-linear raised planting platforms interspersed with shallow, parallel canals filled with water. The elevated planting platforms raise the critical rooting zone of crops such as potatoes, quinoa, or barley above perennially saturated soils. A hypothesized advantage of the raised-field system is that agricultural production can be increased by exploiting the ability of bacteria and *Azolla fililculoides* to fix nitrogen in the canals adjacent to the planting platforms.

To test this hypothesis, nitrogen fixation was estimated *in situ* in the water column of canals, in the soils of planting platforms, in the soils of a dry hillside field, and in the sediments and water column of marshes and totora beds. Nitrogen fixation was significantly greater in the canals of the raised-field systems and in marshes compared to planting platforms and the hillside field. These data support the theory that pre-Hispanic agricultural prosperity of the Andes, as well as other regions of Central and South America, was partially supported by extensive development of raised-field agriculture and probable exploitation of nitrogen fixation.

Key words: *acetylene reduction; altiplano;* Azolla; *Bolivia; nitrogen fixation; raised fields; totora; Tiwanaku.*

THE STUDY OF PRE-COLUMBIAN ANDEAN CIVILIZATIONS offers exceptional insights into human–environment interactions because minor climatic fluctuations render agriculture either possible or untenable, even to the degree that allows civilizations to rise or collapse (Kolata & Ortloff 1996, Binford *et al.* 1997). The emergence, persistence, and subsequent collapse of the Tiwanaku civilization (300 B.C.–A.D. 1100) in the Andean altiplano illustrates remarkable biological and cultural adaptability within a seemingly harsh climatic environment (Kolata 1991, Binford & Kolata 1996).

The ecosystems of the Tiwanaku and Catari River drainage basins near Lake Titicaca constituted the core area of the Tiwanaku civilization (Fig 1). These basins lie at high altitude in the intermontane altiplano (3800–4200 m elev.). The relatively flat basin floors or pampas, are perennially marshy and subject to seasonal inundation and occasional periodic drought. Archaeological evidence and ecological studies indicate that pre-Hispanic agricultural prosperity of this region, as well as other regions of Central and South America, was supported by development of a highly productive and sustainable form of wetlands cultivation: the raised-field agricultural system (Denevan 1970, Darch 1983, Erickson 1988, Carney *et al.* 1996, Kolata & Ortloff 1996).

The Tiwanaku core area contains *ca* 190 km^2 of abandoned raised fields. The raised fields of the Tiwanaku were elevated earthen platforms from 3–10 m wide and up to 200 m long (Kolata 1989, 1991). Seasonally or permanently flooded canals alternated with planting platforms. Approximately 30–60 percent of field areas were planting surfaces. Water in canals was supplied by springs, rivers, or percolating groundwater, depending upon field location. In our study area of the Rio Catari and Rio Tiwanaku basins, the raised-field systems and associated canals, dikes and aqueducts constructed by the Tiwanaku remain visible across the landscape of the pampas (Fig. 2).

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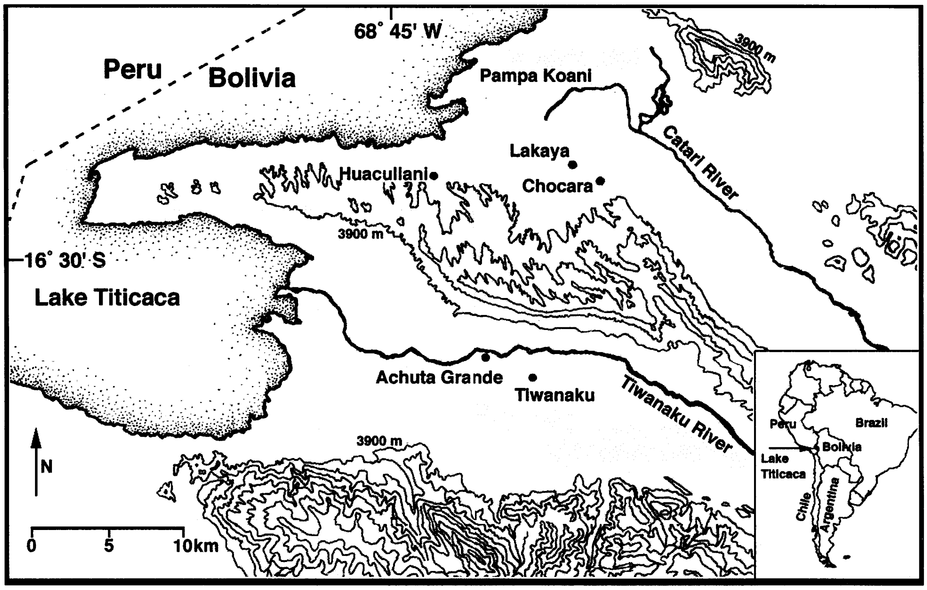

FIGURE 1. The location of the study sites at Lakaya, Chocara, and Achuta Grande in the Catari and Tiwanaku River basins of Bolivia.

experimental raised-field rehabilitation, Binford and Kolata (1996) extrapolate a population in the core area of 285,000–570,000 people during the Tiwanaku V period from 1200–1000 B.P. when raised-field construction reached its peak. This estimate is lower than all but the most conservative estimates of carrying capacity in this environment during antiquity. About 40,000 people now inhabit

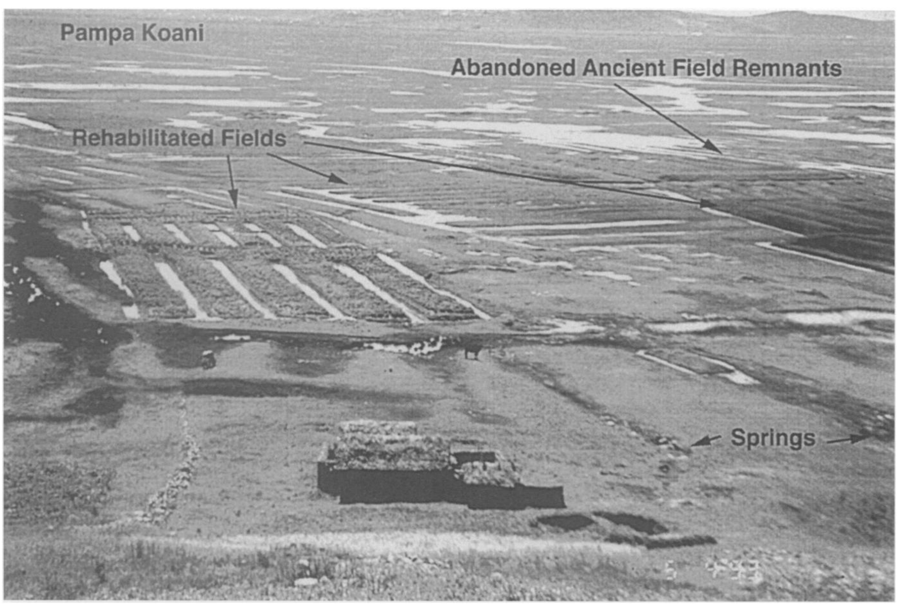

FIGURE 2. Photograph of rehabilitated raised fields at Chocara. Abandoned raised fields are visible to the north and in the distance. Water for the canals of the rehabilitated raised fields emanates from springs at the base of the Serranias Lakayas.

the area, preferentially dry-farming the nutrient-poor, highly erodable soils of steep hillslopes rather than the wet soils of the pampas.

Binford *et al.* (1996) and Carney *et al.* (1993, 1996) hypothesize that diazotrophic organisms living in canals may be an important biological pathway for the introduction of nitrogen into a nitrogen-deficient ecosystem. Soils in the region are very nitrogen poor (Wurtsbaugh *et al.* 1985, Argollo *et al.* 1996, Binford *et al.* 1996). The enhanced productivity of raised fields, however, is maintained by the incorporation of nutrient-rich sediments and aquatic plants from canals into the soils of planting platforms (Gomez-Pompa 1978; Gomez-Pompa *et al.* 1982; Erickson 1985; Kolata 1986; Carney *et al.* 1993, 1996). Diazotrophic species including free-living cyanobacteria, free-living heterotrophic bacteria, and *Anaebaena azollae,* the cyanobacterium associated with the floating water fern *Azolla,* occur in the slack-water canals of raised fields. In this study, we examined the potential for nitrogen fixation in the canals of the raised fields, regional wetlands, and traditional fields.

METHODS

THE STUDY AREA.—Three rehabilitated raised fields in the high altiplano near the villages of Hacienda Lakaya and Hacienda Chocara in the Rio Catari basin, and Achuta Grande, northwest of Tiwanku in the Rio Tiwanaku basin, were chosen as principal study sites (Fig. 1). The sites at Lakaya and Chocara were located *ca* 12 km southeast of the Lake Titicaca shoreline. They are within 300 m of the northern edges of the two villages with the same names(on the margin of the Pampa de Cohani Pata). The villages lie along the margins on the slopes of a range of small hills, the Serranias Lakaya Pata. The Achuta Grande site was *ca* 11 km east of Lake Titicaca and 4 km west-northwest of the village of Tiwanaku near the center of the Iitiri Pampa. The pampas proximate to these villages are perennially marshy this is attributable to permanent subterranean groundwater seepage from underlying aquifers and permanent surface flow from springs that emerge onto the land surface from the base of the hills of the Serranias Lakaya, or from nearby rivers such as the Rio Tiwanaku at Achuta Grande (Ortloff & Kolata 1996). The merging of these low-gradient aquifers and the surface of the pampas creates pools of standing water and marshes on the landscape.

The three rehabilitated raised fields are described as the Lakaya site (3812 m elev.; UTM Zone 19 coordinates: 8182386 N, 537262 E), the Chocara site (3812 m elev.; UTM coordinates: 8181705 N, 539023 E), and the Achuta Grande site (3816 m elev.; UTM coordinates: 8168900 N, 534570 E). The rehabilitated fields are small-scale reconstructions of the extensive raised-field systems built by the Tiwanaku (see Kolata *et al.* 1996 for a detailed summary of rehabilitated raised fields in this area). The raised field at Achuta Grande was at the southeastern end of a *ca* 10-ha complex of fields being currently maintained and studied by faculty and students at the Unidad Academica Campesina of the Bolivian Catholic University. Data including *in situ* assays of nitrogen fixation via acetylene reduction, soil characteristics, soil and water temperature, and identification of species present at the study sites were gathered from: (a) canals, (b) planting platforms, (c) marshy areas fed by springs or percolating groundwater within 20 m of the raised fields, and (d) a steep, dry hillside field above the Chocara site that is typical of fields currently used for cropping in preference to raised fields (3840 m elev.; UTM coordinates: 8181574 N, 538954 E). This latter site will be referred to as the dry hillside field. Similar measurements were made in a large shoreline bed of the regionally important totora plants (Raynal-Roques 1992) located at the eastern edge of Lake Titicaca on the western edge of Pampa de Cohani Pata (3811 m elev.; UTM coordinates: 8187820 N, 533232 E). This site will be referred to as the totora bed, and could only be sampled once during the dry period. Data were gathered from all locations during the austral winter between 30 August and 2 September 1995 and in the summer 10–16 February 1996 (with the exceptions noted below).

At the Lakaya site, the rehabilitated raised-field system consisted of eight planting platforms and nine canals bordered by two end canals laid out in a rectanglar manner. It was representative of other raised fields in the area. A channelized stream emanating from a spring near the margin of the pampas supplied water to the raised-field system. Mean water depth in canals was 25 ± 4 cm (N = 10). Although we gathered data from this raised-field in the winter of 1995, we were excluded from from this site in the summer of 1996 because of insurmountable, local political difficulties. The beds were fallow and not in production during the previous growing season but previously had been cropped.

The rehabilitated raised-field at the Chocara site was similar but composed of six canals and five planting platforms oriented in the same compass directions as the raised fields at Lakaya. The overall dimensions of the raised-field were *ca* 61-m long and 34-m wide, including the end canals. The mean width of the canals was 2.1 m (N = 10) and the mean width of the planting platforms was 4.5 m (N = 10). The raised fields were fallow in 1995 and 1996. The system was not connected to its nearby water supply because the system was not in production, however, the two canals on the outside of the system were partially flooded during both study periods. Mean water depth was 28 ± 5 cm (N = 6). The three center canals and end canals were dry. A spring and marshy areas immediately south and west of the site were the source of water for the raised-field system. Data were obtained from this site in both the winter of 1995 and the summer of 1996.

The Achuta Grande site, at the eastern end of a large complex of raised fields, was partially excavated from a river and spring-fed marshy swale that served as a source of water for the raised fields in this area. The study site included a short end canal with three canals abutting it at right angles. Canal dimensions were similar to the Lakaya and Chocara sites. The end canal was 25-m long, canals were 2.2-m wide (N = 12), and water depth was 35 ± 6 cm (N = 12). The system was oriented in an east-west direction. In contrast to the raised fields at Lakaya and Chocara, this complex of beds was in full production and planted with potatoes. This raised field and surrounding area was sampled in the summer of 1996.

FIELD MEASUREMENTS.—Nitrogen fixation associated with plants and soils/sediments were estimated simultaneously by the acetylene-reduction (AR). *In situ* open-ended system assays were performed as described by Eckardt and Biesboer (1988a) in both canals and dry fields. Thick-walled polyvinyl chloride water pipes (10-cm internal diameter [i.d.] × 60 cm) with gas-tight caps and removable rubber septa were sharpened at their ends and placed into the sediments of canals or placed into the dry soils of planting platforms and dry fields by excavating to a minimum depth of 20 cm.

Cylinders were placed easily into the saturated organic sediments of canals, marshes, and totora bed. In the canals and marshy areas, cylinders were placed over floating, emergent, or submerged vegetation. It was impossible to drive cylinders into the dry soils of planting platforms or into the extremely dry, rocky soils of upland fields. In these cases, soils were excavated to a minimum depth of 20 cm to accommodate a sampling cylinder. The bottom of the excavation was saturated with freshwater and a plastic sheet placed over the end of the cylinder to provide a gas-tight seal. Excavated soil was placed into the cylinder.

Closed vessel assays were performed when it was obvious that large, partially submerged or floating algal colonies and floating *Azolla* plants were present in the canals and contributing to nitrogen fixation in the water column. For these closed system assays, algal colonies or floating *Azolla* plants were harvested from the flooded canals by carefully submerging shallow, clear plastic 1.5-liter, gas-tight assay containers (fitted with rubber septa underneath the colonies or populations) to capture a portion of the colony representative of the surface area occupied by these organisms. *Lemna* plants (estimated at *ca* 5% total surface area) were usually mixed with the *Azolla*. A control container was filled only with canal water. Estimates of nitrogen fixation in the light were made *in situ* by floating the containers at the shaded edge of a canal where they would not be heated by solar radiation during incubation.

At the totora site, cylinders were placed over totora plants near Lake Titicaca in an area where the lake had recently receded, leaving the totora exposed ("*totora seco*" or dry totora). These plants varied from *ca* 2–3 m in height and were truncated at 10 cm from the sediment surface prior to placing an open-ended sampling cylinder over them. The totora formed a monospecific stand in this area although a small number of individuals of the submerged aquatic species, *Myriophyllum quitense* and *Elodea potamogeton,* were associated with the totora in the flooded areas of the sampling site.

Cylinders or assay vessels were carefully set at the various sites as described in Table 1. A total of 87 open-ended and closed vessel assays, including controls, were performed during the course of this research.

Various plants were present at the study sites as indicated under "field notes" in Table 1. Algal taxa are only identified as algal mats or free-living unicellular forms, and by microscopic inspection were found to be predominantly green algae and cyanobacteria (principally *Anabaena, Nostoc,* and *Oscillatoria* spp.). Representative higher plants were collected, pressed, and identified at the Herbario Nacional de Bolivia in La Paz, Bolivia. These taxa (referred to only by generic names in the tables and text of this paper) included: *Azolla filiculoides* Lam.; *Elodea potamogeton* (Bert.) Espin; *Festuca orthophylla* Pilger; *Hydrocotyle ranunculoides* L.f.; *Lemna gibba* L.; *Myriophyllum quitense* H.B.K.; *Potama-*

geton strictus Ruiz & Pavon; *Schoenoplectus tatora* (Kunth) Palla; and an unidentified *Chara* sp. and *Juncus* sp.

Headspaces of sampling cylinders were adjusted to a volume of 1 liter (± 10 ml; range = 0.89–1.13 liters) by careful positioning of each cylinder. Ten percent of the gaseous phase of the headspace was replaced with acetylene generated in the field from calcium carbide. Twelve ml gas samples were collected at 0, 6, 12, and 24 h and stored in 15-ml Vacutainers® (silicon coated; 15 ml) for subsequent chromatographic analysis in the laboratory of one author (DDB). The ends of Vacutainers® were wrapped in teflon tape and kept cool in styrofoam containers until analysis. Control cylinders were not injected with acetylene and were placed over similar soils or plants as checks for endogenous ethylene production.

Water and soil temperature (at 10 cm below the surface) were recorded at each sampling site using a mercury field thermometer. Soils were collected using a handheld soil recovery probe (1.8-cm diam.) inserted to a depth of 20 cm. Three cores were taken at each site within 1 m of each sampling cylinder. These samples were thoroughly homogenized in the field and a 100-g subsample collected for analysis. Particle size analysis was done using a Lamotte Soil Texture Unit (Forestry Suppliers, Inc., Jackson, Mississippi). Concentrations of nitrate and ammonium in canal water and soil pore water were determined in the field using the cadmium reduction method for nitrate and the Nessler reagent method for ammonium. Water pH was determined using a field pH meter. Sediment and soil pH was determined using the standard soil:distilled water (v:v) mixture. Samples were analyzed for other parameters in the laboratory.

Laboratory analyses.—Gas samples for acetylene reduction assays were analyzed using a Hewlett-Packard 5840 A gas chromatograph fitted with a flame ionization detector and a 100/120-mesh Porapak R column (2 mm i.d. $\times$ 1.0-m stainless steel). Nitrogen was used as the carrier gas at 40 ml/min. Analyses were performed isothermally at 60°C. Ten Vacutainers® were injected with a known mixture of acetylene and ethylene (80/20%, respectively) prior to leaving the United States for Bolivia. Analysis of these standard mixtures after returning to the United States indicated that leakage of Vacutainers® was less than 1 percent during transport. Calculations were performed according to Turner and Gibson (1980). Detection limits for ethylene were 500 nmol.

A Carlo Erba C/N/S analyzer was used to determine the percentage of carbon and nitrogen in soils.

RESULTS

Daily water and soil temperatures varied widely during the winter sampling at Lakaya and Chocara. Soil temperatures ranged from 6°C in the early morning to 30°C at 10 cm below the soil surfaces of planting platforms or hillside fields in the late afternoon as sunlight warmed the soil surface. Water temperatures ranged from 0°C in the morning to 15°C in the afternoon in protected areas of canals exposed to direct sunlight. A 1 to 2-cm layer of ice formed around sampling cylinders and the vessels floated overnight in canals for the *in situ* assays.

In comparison, water and soil temperatures were higher but less variable during the summer growing season at Chocara and Achuta Grande. At both sites, canal and marsh water temperatures ranged from 20°C in the early morning to 27°C in late afternoon. Soil temperatures at 10 cm below the surface varied from 21°C in the early morning to 24°C in the late afternoon. Soils of the planting platforms and the traditional hillside fields were often cooled by light rains that fell nearly daily. Annual mean air temperatures are 9°C with a daily variation of *ca* 12°C (Boulange & Aquize Jaen 1981).

Sediments of marshes, canals, and totora beds were composed of finally sedimented, highly reduced, gray marly alluvial clays to a depth of 60 cm, often overlaid by several centimeters of flocculent organic material and plant detritus. Table 2 summarizes some important soil parameters for platform soils, canal sediments, marsh sediments adjacent to raised fields, hillside soil, and sediments of the totora bed. Soil and sediment pH ranged between 7.2 and 7.8. Percent carbon of soils was (in decreasing order): marshes > canals > planting platforms > totora bed > hillside. Percent nitrogen of soils was observed to be in decreasing order: canals >marshes> totora bed>planting platforms>hillside. Soils of the planting platforms, excavated from the adjacent canals, were often encrusted with a layer of visible salts left after evaporation of surface moisture. Particle size distribution was roughly similar for platform soils (excavated from canals), canal sediments, marsh sediments, and the totora bed. All were high in clay and silt. In contrast, the brown, oxidized soil of the hillside was composed of 50 percent rocks and

TABLE 1. *Field sites, type and number of assays performed in the field, location of assays, and field notes concerning flora present at the site of each assay.*

Site	Type/no. of assays—assay designation	Assay location	Field notes
Lakaya	Cylinder/3-a	Flooded canal	100 percent of water surface covered with *Azolla*; plants crowded to several layers thick; filamentous algae mats or plant roots not present in water or sediments.
	Cylinder/3-b	Flooded canal	50 to 100 percent of water surface covered with *Azolla* as a single layer; some filamentous algae present in water column.
	Cylinder/3-c	Flooded canal	<10 percent of water surface covered with *Azolla*; some filamentous algae present in water column.
	Cylinder/2	Flooded canal	Controls; *Azolla* or filamentous algae not present.
	Cylinder/3-d	Planting platform	Cylinders located 6 m apart at center of platform; surface of the soil bare with slight saline crust; crop residue present.
	Cylinder/1	Planting platform	Control.
	Closed assay/3-e	*In situ* in canal	Closed assay vessel (1.5 liter) containing *Azolla* and *Lemna*; plants very crowded into several layers at water surface.
	Closed assay/1	*In situ* in canal	Control.
Chocara	Cylinder/6-a	Flooded canal	Dense algal mat in water column; sediments support dense population of *Chara*; *Juncus* roots in sediments; (3 sample cylinders in winter 1995 and 3 in summer 1996).
	Cylinder/2	Flooded canal	Control.
	Cylinder/6-b	Marsh upstream of the raised fields	Filamentous and unicellular algae in water column; 50–75 percent of water surface covered with *Azolla*; roots of *Juncus* and *Hydrocotyle* present in sediments; (3 sample cylinders in winter 1995 and 3 in summer 1996).
	Cylinder/2	Marsh upstream of the raised fields	Control.
	Cylinder/6-c	Planting platform	Cylinders located 2 m apart at center of platform; surface of the soil bare with slight saline crust; (3 sample cylinders in winter 1995 and 3 in summer 1996).
	Closed assay/3-d	*In situ* in canal	Closed assay vessel (1.5 liter) containing *Azolla*.
	Closed assay/1	*In situ* in canal	Closed assay vessel (1.5 liter) containing *Azolla*.
	Cylinder/6-e	Dry, hillside field	Steep hillside above Chokara; two assays in fallow field in soil containing some crop residue; one assay at field edge 3 m away; two assays 10 m away from field in uncultivated area adjacent to *Festuca* plants; (3 sample cylinders in winter 1995; and 3 in summer 1996).
	Cylinder/2	Dry, hillside field	Control set in field.

TABLE 1. *Continued.*

Site	Type/no. of assays—assay designation	Assay location	Field notes
Achuta Grande[a]	Cylinder/9-a	Flooded canals	Three assays in canals where filamentous algal mats were present; five assays at edge of canal with *Hydrocotyle* roots in cylinders, blue-green bacteria mat near root surfaces; one assay in adjacent marsh dominated by *Hydrocotyle*; *Azolla* not present in the 10-ha Achuta Grande complex.
	Cylinder/3-b	Flooded canals	Algae or plants not visibly present in water column.
	Cylinder/3	Flooded canal	Controls.
	Cylinder/3-c	Planting platform	Potatoes under cultivation; potato roots present in cylinder.
	Cylinder/1	Planting platform	Control.
	Closed assay/3-d	*In situ* in canal	Closed assay vessel (1.5 liter) containing filamentous algal mats.
	Closed assay/l	*In situ* in canal	Control.
Totora bed	Cylinder/9-a	Totora bed	30-m^2 area; three cylinders surrounding totora plants in standing water with *Elodea, Potamageton* in the water column; three cylinders over tortora plants in sediments; three cylinders over totora plants in drier but saturated sediments; all totora plants truncated at 10 cm above sediment/water surface; *Azolla* and filamentous algal mats not present at this site; sampled only in winter 1995.
	Cylinder/2	Totora bed	Controls: two cylinders; one over truncated totora in saturated sediment and one in dry site over sediment only.

coarse sand and showed the lowest content of fine sands, silts, and clay.

Table 3 summarizes mean values for μmol of ethylene produced by acetylene reduction (AR) assays for flooded canals, soils of planting platforms, marshes immediately adjacent to the raised-field systems, the dry hillside field, the tortora bed, and closed vessel assays of filamentous algal mats and *Azolla.* AR activity per cylinder or closed vessel assay as function of time (*i.e.*, ethylene reduced at 6,

TABLE 2. *Summary of soil data for the different sites at Lakaya, Chocara, Achuta Grande, and the totora bed at the eastern edge of Lake Titicaca. Means for pH, percent C, and percent N are values (± SD) for nine combined soil samples taken from platforms, canals, and marsh sediments across all study sites. For the single hillside site and totora bed, values are the means of three homogenized soil and sediment samples. Particle size analysis was performed once for combined soil samples from each site.*

	Soil parameter						
Sample	pH	percent C	percent N	Rocks/ coarse sand	Fine sand	Silt	Clay
Platform soils	7.2 ± 0.1	3.58 ± 0.74	0.31 ± 0.04	0	22	22	56
Canal sediments	7.4 ± 0.3	5.20 ± 0.58	0.81 ± 0.77	0	24	19	57
Marsh sediments	7.3 ± 0.1	7.61 ± 0.55	0.63 ± 0.05	0	15	24	61
Hillside soil	7.3	1.40	0.14	50	11	4	35
Totora bed sediment	7.3	3.49	0.39	0	10	27	63

TABLE 3. *Mean values for nitrogen fixation as estimated by acetylene reduction (AR). Values are calculated as μmol of ethylene produced/m²/24 h (± SD) for comparison to other values in the literature. Endogenous ethylene production was not detectable in control cylinders or assay vessels. Refer to Table 1 for corresponding field notes for each cylinder or assay set. NA = Not Assayed (see text).*

Site	Type/no. of assays—assay designation	Assay location	[1]μmol ethylene/m²/24 h Winter 1995	Summer 1996
Lakaya	Cylinder/3-a	Flooded canal	146.0 ± 67.9 a	NA
	Cylinder/3-b	Flooded canal	55.6 ± 48.4 b	NA
	Cylinder/3-c	Flooded canal	13.8 ± 0.9 c	NA
	Cylinder/3-d	Planting platform	0.5 ± 0.3 d	NA
	Closed assay/3-e	Canal *in situ—Azolla*	537.6 ± 280.2 e	NA
Chocara	Cylinder/6-a	Flooded canal	27.2 ± 8.9 f	39.2 ± 22.6 i
	Cylinder/6-b	Marsh	58.7 ± 39.9 b	90.4 ± 34.2 g
	Cylinder/6-c	Planting platform	2.0 ± 0.1 h	2.5 ± 0.2 h
	Closed assay/3-d	Canal *in situ—Azolla*	NA	186.1 ± 55.9 a
	Cylinder/6-e	Dry, hillside field	trace	trace
Achuta Grande	Cylinder/9-a	Flooded canal	NA	28.0 ± 10.2 f
	Cylinder/3-b	Flooded canal	NA	3.2 ± 2.7 h
	Cylinder/3-c	Planting platform	NA	0.62 ± 0.2 d
	Closed assay/3-d	Canal *in situ*—algal mats	NA	44.9 ± 19.7 b
Totora bed	Cylinder/9-a	Totora bed	11.8 ± 8.8 c	NA

[1] Means followed by the same letter are not significantly different according to Fisher's PLSD multiple comparison test at a 95 percent level of significance.

12, and 24 h) shows typical rate curves for these types of assays (data not illustrated; Biesboer 1984a, b; Eckardt & Biesboer 1988a, b; Biesboer *et al.* 1998). High variability is expected in assays of these types because unknown numbers and species of various nitrogen fixing autotrophs and heterotrophs are associated with the plants in each assay cylinder. *Azolla filiculoides* is an important nitrogen fixing species in the raised-field agricultural system. Cylinders or closed assay vessels containing *Azolla* plants exhibited the highest accumulations of ethylene during the 24-h assay periods. The highest detectable activities occurred in the Lakaya cylinders set (3-a; from a canal in which the surface was 100 percent covered with several layers of floating plants) and in closed assay vessels containing *Azolla* (incubated *in situ* at Lakaya [3-e] during the winter of 1995 and at Chocara (3-d) during the summer of 1996).

The importance of the number of *Azolla* plants present on the surface of a canal is made readily apparent from values obtained for the series of cylinders placed in canals at Lakaya in which the surfaces were covered with a crowded layer of plants, a single layer of plants, or <10 percent of the water surface (Lakaya 3-a, 3-b, 3-c, respectively). Ethylene production decreased by an order of magnitude from 146.0 to 55.6 to 13.8 μmol ethylene/m²/24 h. The closed vessel assay containing *Azolla* incubated *in situ* at Lakaya (3-e) is illustrative of the highest AR rates in the light that might be encountered in canals that support dense populations of *Azolla*. The *Azolla* for this assay was collected from a narrow, deep, and well-protected (from freezing) canal that supported a luxurious 2 to 4-cm thick mat of *Azolla* mixed with some *Lemna*. This canal presented ideal growing conditions for this species but was unusual because canals and marshes generally had water surfaces covered by a single layer of plants.

The effects of temperature on acetylene reduction are shown by comparing values for AR in canals and marshes for the winter and summer at Chocara. As mentioned, water temperatures varied from 0°C in the morning to 15°C in the afternoon in protected areas of canals exposed to direct sunlight in the winter and from 20°C in the morning to 27°C in the summer. At Chocara, increases in AR occurred from winter to summer (27.2–39.2 μmol ethylene/m²/24 h in canal assays (6-a) placed over algal mats; 58.7–90.4 μmol ethylene/m²/24 h in marsh assays (6-b) in which *ca* 50–75% of the water surface was covered by *Azolla*). Populations of *Azolla (i.e.,* their surface coverage and density) and the size and location of algal mats in the water column appeared to remain stable between winter of 1995 and summer of 1996.

Algal mats, which by microscopic inspection were composed of both filamentous green and blue-green taxa, also contributed to AR activity in canals but at lesser rates than in canals that support *Azolla.* Canal assays at Chocara set over algal mats (6-a) in both winter and summer (27.26 and 39.23 μmol ethylene/m^2/24 h, respectively), and closed vessel assays at Achuta Grande (3-c; 28.02 μmol ethylene/m^2/24 h) showed considerable amounts of AR activity but always at rates lower than for assay cylinders or vessels in which the water surface was covered 50 percent or more by *Azolla.*

Cylinders not containing *Azolla,* visible mats of algae, or higher plant roots (Achuta Grande; 3-b) showed minimal amounts of nitrogen fixing activity. Likewise, planting platforms and the dry, rocky soils of the hillside field supported little or no AR activity. The soils of planting platforms at Lakaya (3-d), Chocara (6c), and Achuta Grande (3-b) reduced 2.50 μmol ethylene/m^2/24 h or less. Ethylene was detectable only in the 24-h assay of sampling cylinders placed on planting platforms. The soils of the traditional dry field in the hills above Chocara exhibited only a trace amount of AR activity in both winter and summer.

Acetylene reduction activity also occurred in association with totora plants in the large totora bed (9-a) found near the shores of Lake Titicaca. The mean value of 11.8 μmol ethylene/m^2/24 h was intermediate to the values found for the *Azolla* assays and algal mats in the canals of the raised-field systems.

Nitrate and ammonium concentrations of canal water and soil pore water were also very low. Nitrate was either not detectable or found to be $<$ 1.0 mg/liter (N = 20). Ammonia was consistently found to be between 3–6 mg/liter for the saturated soils of canals and marshes (N = 20).

DISCUSSION

The soils of the often steep upland hills are xeric, highly erodable, and composed of 50 percent rocks and coarse sand (Table 2). They are poor sites for nitrogen fixation. In contrast, the pampas of the Rio Tiwanaku and Rio Catari basins have soils composed of fine fluvial and lacustrine deposits that are often saturated because of periodic inundation by rains, percolating groundwater, standing water, springs, and large and small streams. Their flora is dominated by aquatic vegetation. These wetland areas act as catchments for nutrients and particulates eroding from the surrounding hills, with the wet soils and standing water favoring free-living diazotrophic, autotrophic, and heterotrophic microorganisms, and the microorganisms associated with the rhizosphere of aquatic plants (Tjepkema & Evans 1976).

Soils of the Andes are especially nitrogen-poor (Vincent *et al.* 1984, 1985; Wurtsbaugh *et al.* 1984, 1985; Argollo *et al.* 1996). The dry, rocky, poorly organic soils of the upland noncultivated and cultivated hills in the Catari basin are high in phosphorus but very low in total nitrogen (Binford *et al.* 1996). In contrast, the wet organic sediments of canals between raised fields exhibit similar levels of phosphorus but enhanced levels of nitrogen (Binford *et al.* 1996; Table 2). In this study, the canal sediments exhibited increased amounts of total nitrogen compared to other soils and sediment, most probably because of the large numbers of nitrogen fixing organisms present in the canal system (Table 2). Ammonium, as expected, was the principal species of nitrogen found in the reduced sediments of canals.

Enhanced levels of carbon and nitrogen in the canals of raised fields occur for two reasons: (1) the fields act as detention basins in the watershed that slow or stop the movement of dissolved and particulate-bound nutrients from the uplands to the lake basin (Carney *et al.* 1993); and (2) the canals afford an ideal habitat for the growth of nitrogen fixing organisms and aquatic angiosperms (such as totora) that show considerable nitrogen fixation associated with their root systems. Although different organisms contribute to the importation of newly fixed nitrogen into the canals of the raised-field system, *Azolla,* via its symbiont *Anabaena azollae,* is the most important nitrogen-fixing organism (Table 4).

The contribution of *Azolla* to nitrogen fixation in canals of a single field can be estimated by simple calculation. Using the conservative value of 55.6 μmol ethylene/m^2/24 h (Table 4; Lakaya Cylinder assays/3-a; assayed over surfaces typically covered by a single layer of *Azolla* during the winter) and using the standard ratio of 3 mol C_2H_4 reduced:1 mol N_2 fixed, *Azolla* that covers the surface of a canal in a single layer can fix 0.25 g N/m^2/d or 2.5 kg N/ha/d. This value of 2.5 kg N/ha/d compares favorably to other values reported for *Azolla filiculoides* (range = 1.5–2.6 kg/N/ha/d; Nierzwicki-Bauer 1990). On an annual basis, *Azolla* would fix 2.5 kg N/ha/d × 365 d or 912.5 kg/ha/yr. Estimates would be higher or lower depending on the density of the population of *Azolla* and/or presence of other cyanobacteria in individual canals. Estimates could be made also for nitrogen gained per day via *Azolla* for a typical rehabilitated

TABLE 4. *Estimates of nitrogen fixed within the raised-field system as kg N/ha/d and kg N/ha/yr. The table uses conservative values for AR gathered in the winter of 1995 (see Tables 1 and 2) to calculate kg N/ha/d for: (1) canals covered with a single layer of* Azolla; *(2) dense algal mats in the water column; (3) small numbers of* Azolla *on the water surface and presence of some filamentous algae in the water column; and (4) the soils of planting platforms.*

Order of importance	Assay	μmol ethylene/ m^2/24 h (acetylene reduction)	kg N/ha/d	kg N/ha/yr
1	Flooded canal; *Azolla* covers surface (Lakaya cylinder/3-b, 1995)	55.6	2.5	912.5
2	Flooded canal; dense algal mats in water column (Chocara cylinder/3-a, 1995)	27.2	1.2	438.5
3	Flooded canal; small number of *Azolla* and algae present (Lakaya cylinder/3-c, 1995)	13.8	0.6	219.0
4	Planting platform (Chocara cylinder/6-c, 1995)	2.0	0.09	32.8

raised field. The total area of the canals in the rehabilitated raised field at Lakaya is 400.3 m^2. Total nitrogen fixed in the entire raised-field system at Lakaya would be 400.3 $m^2 \times 0.25$ g/ m^2/24 h $\times$ 365 d/yr or 36.5 kg N on an annual basis if the canals of the raised-field system supported a single layer of *Azolla.*

Table 4 compares the combinations of nitrogen fixing organisms that might be present in a canal and their potential to fix nitrogen. Both *Azolla* and blue-green bacteria, when present as mats in the water columns, are the principal contributors to nitrogen gains from nitrogen fixation in the raised-field agricultural system. As expected, little nitrogen fixation occurs on the drier, elevated soils of the planting platforms.

Although this paper focuses on the nitrogen fixation occurring in the raised-field system, it is obvious from AR assays performed in adjacent marshes and in the totora bed located on the shores of Lake Titicaca that the wetlands of the pampas are regionally important for bringing additional nitrogen into this nitrogen depauparate ecosystem. Thirty-five percent and 12 percent of the Catari River valley and Tiwanaku River valley, respectively, are designated as permanent wetlands (Binford & Kolata 1996). The marsh adjacent to the raised-field system in Chocara that supported a population of *Azolla* showed increased levels of AR in both winter and summer (Table 3). Plants in the totora beds also showed values of AR that were slightly less than in some of the cylinder assays for canals during the same time period (Table 3: Lakaya Cylinder/3-c and tortora bed). Totora is one of the most important sources of fodder for livestock in the region.

In 1995, the totora beds were accessible from the shoreline because of low water levels in Lake Titicaca. Many villagers pastured their cattle in the dry totora beds. Totora is also harvested and often transported many kilometers from the lake edge to be dried and stockpiled for later use. Additionally, it is frequently transplanted into the canal edges of raised fields or into marshy areas near individual households where it becomes a local cultivated source of totora.

Some evidence exists for the increased productivity of raised fields as evidenced by contemporary land use patterns in the altiplano. Local farmers crop hillside fields about every five or six years. In contrast, rehabilitated raised fields are cropped annually by some farmers. Kolata *et al.* (1991) compared three agricultural practices: traditional cultivation without commercial fertilizers; traditional cultivation with the addition of commercial fertilizers; and experimental raised fields. On 12 cultivated parcels of experimental, rehabilitated raised fields in the 1987–88 season, potato yields were twice the yield of traditional fields supplemented with chemical fertilizers, and over seven times the yield of unimproved traditional cultivation. The pampas, however, are rarely used for growing crops. Approximately 10 percent of the topographically higher and drier areas of the Bolivian pampas are farmed, with the rest of the area being devoted to pasturage of livestock.

Although it is clear that raised fields can increase agricultural productivity, it is not possible at this time to determine the maximum annual nitrogen gain to crops grown on raised fields via the growth and incorporation of *Azolla,* algae, or other aquatic plant species into the beds of the planting platforms. Many factors will influence the use of *Azolla* and algae as green manures in the altiplano, including the nitrogen requirements of local crops, presence or absence of other important soil nutrients, loss of nitrogen via denitrification and other pathways such as leaching, annual growth limitations, and periodic harvesting of *Azolla* for incorporation into planting platforms. Agronomically meaningful nitrogen fixation has been defined as > 5 kg N/ha for the aquatic crop of rice (Ventura & Watanabe 1983). *Azolla* or blue-green bacteria, however, have not been historically exploited as an alternative nitrogenous fertilizer in the high altitude tropics as they have been in lowland regions of the tropics (Singh 1979, Roger & Kulasooriya 1980, Reynaud 1982, Watanabe 1982, Ye 1984, Kikuchi *et al.* 1985, Kumarasinghe & Eskew 1993).

Several important conclusions can be made from this study of nitrogen fixation in the raised-field system, as practiced extensively by the Tiwanku culture in the past and only sporadically in rehabilitated raised fields in modern times. The Tiwanaku farmed the pampas intensively by utilizing 190 km^2 of its soil surface to support a population of 285,000 to 570,000 people until a 200-year drought caused the collapse of their civilization (Kolata & Ortloff 1996, Binford *et al.* 1997). Their economic success as a civilization was due partially to the exploitation of raised fields to biogeochemically capture limiting plant nutrients. Canals and their associated planting platforms can realize a major net gain in nitrogen over time as nitrogen is fixed by diazotrophic organisms in canals and taken up by other plants living in the same aquatic environment. These organisms and aquatic plants growing in canals, if efficiently managed, can provide a nitrogen-rich green manure for incorporation into the soils of planting platforms (Kolata 1991).

Based on the findings of this study, we recommend that farmers using raised-field systems keep canals filled with water throughout the year. Canals that become dry because of low rainfall or diversion of water sources for other purposes, kill the populations of nitrogen fixing organisms and aquatic plants inhabiting the canals.

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LITERATURE CITED

ARGOLLO, J., L. TICCLA, A. L. KOLATA, AND O. RIVERA. 1996. Geology and soils of the Tiwanaku and Catari River basins. *In* A. Kolata (Ed.). Tiwanaku and its hinterland: archaeology and paleoecology of an Andean civilization. 1. Agroecology, pp. 57–88. Smithsonian Institution Press, Washington, DC.

BIESBOER, D. D. 1984a. Seasonal variation in nitrogen fixation, associated microbial populations, and carbohydrates in roots and rhizomes of *Typha latifolia* L. (Typhaceae). Can. J. Bot. 62: 1965–1967.

———. 1984b. Nitrogen fixation associated with natural and cultivated stands of *Typha latifolia* L. (Typhaceae). Am. J. Bot. 71(4): 505–511.

———, F. ESTEVES, AND A. E. PRAST. 1998. Nitrogen fixation associated with *Typha domingensis* Pers. in Lagoa Imboacica, a coastal lagoon of Rio de Janeiro state, Brazil. Verh. Intern. Verein. Limnol. 26: 1412–1417.

BINFORD, M. W., AND A. L. KOLATA. 1996. The natural and human setting. *In* A. Kolata (Ed.). Tiwanaku and its hinterland: archaeology and paleoecology of an Andean civilization. 1. Agroecology, pp. 89–108. Smithsonian Institution Press, Washington, DC.

———, M. BRENNER, AND B.W. LEYDEN. 1996. Paleoecology and Tiwanaku agroecosystems. *In* A. Kolata (Ed.). Tiwanaku and its hinterland: archaeology and paleoecology of an Andean civilization. 1. Agroecology, pp. 89–108. Smithsonian Institution Press, Washington, DC.

———, A. L. KOLATA, M. BRENNER, J. W. JANUSEK, M. T. SEDDON, M. ABBOTT, AND J. H. CURTIS. 1997. Climate variation and the rise and fall of an ancient Andean civilization. Quatern. Res. In press.

BOULANGE, B., AND E. AQUIZE JAEN. 1981. Morphologie, hydrographie et climatologie du lac Titicaca et de son bassin versant. Revue d' Hydrobiologie Tropicale 14: 269–287.

CARNEY, H. J., M. W. BINFORD, AND A. L. KOLATA. 1996. Nutrient fluxes and retention in Andean raised-field agriculture: implications for long-term sustainability. *In* A. Kolata (Ed.). Tiwanaku and its hinterland: archaeology and paleoecology of an Andean civilization. 1. Agroecology, pp. 170–180. Smithsonian Institution Press, Washington, DC.

———, ———, ———, R. R. Marin, and C. R. Goldman. 1993. Nutrient and sediment retention in Andean raised-field agriculture. Nature 364: 131–133.

Darch, J. P. 1983. Drained fields of the Americas. British Archaeological Reports, International Series no. 189. Oxford, England.

Denevan, W. M. 1970. Aboriginal drained-field cultivation in the Americas. Science (Wash DC.) 169: 647–654.

Eckardt, N., and D. D. Biesboer. 1988a. A survey of nitrogen fixation (AR) associated with *Typha* in Minnesota. Can. J. Bot. 66: 2419–2423.

———, and ———. 1988b. Ecological aspects of nitrogen fixation (AR) associated with plants of a Minnesota wetland community. Can. J. Bot. 66: 1359–1363.

Erickson, C. L. 1985. Applications of prehistoric Andean technology: experiments in raised-field agriculture, Huatta, Lake Titicaca: 1981–1982. *In* I. Farrington (Ed.). Prehistoric intensive agriculture in the tropics, Part 1, pp. 209–232. British Archaeological Reports, International Series 232(I), Oxford, England.

———. 1988. Raised-field agriculture in the Lake Titicaca basin: Putting ancient Andean agriculture back to work. Expedition 30 (3): 8–16.

Gomez-Pompa, A. 1978. An old answer to the future. Mazingira 5: 50–55.

———, H. L. Morales, E. Jiménez Avilla, and J. Jiménez Avilla. 1982. Experiences in traditional hydraulic agriculture. *In* K. Flannery (Ed.). Maya subsistence, pp. 327–342. Academic Press, New York, New York.

Kikuchi, M., I. Watanabe, and L. D. Haws. 1985. Economic evaluation of *Azolla* use in rice production. *In* Organic matter and rice, p. 569. International Rice Research Institute, Los Banos, the Philippines.

Kolata, A. L. 1986. The agricultural foundations of the Tiwanaku state: a view from the heartland. Am. Antiq. 51: 748–762.

———. 1989. Introducción: Objectivos y estrategias de la investigación. *In* A. L. Kolata (Ed.). Arqueología de Lukurmata, Vol. 2, pp. 13–40. Instituto Nacional de Arqueología y Ediciones Puma Punku, La Paz, Bolivia.

———. 1991. The technology and organization of agricultural production in the Tiwanaku state. Lat. Am. Antiq. 2 (2): 99–125.

———, and C. R. Ortloff. 1996. Agroecological perspectives on the decline of the the Tiwanaku state. *In* A. Kolata, (Ed.). Tiwanaku and its hinterland: Archaeology and paleoecology of an Andean civilization. 1. Agroecology, pp.181–201. Smithsonian Institution Press, Washington, DC.

———, O. Rivera, J. C. Ramírez, and E. Gemio. 1996. Rehabilitating raised-field agriculture in the southern Lake Titicaca basin of Bolivia. *In* A. Kolata, (Ed.). Tiwanaku and its hinterland: archaeology and paleoecology of an Andean civilization. 1. Agroecology, pp. 203–230. Smithsonian Institution Press, Washington, DC.

Kumarasinghe, K. S., and D. L. Eskew (Eds.). 1993. Isotopic studies of *Azolla* and nitrogen fertilization of rice, pp. 1–145. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Nierzwicki-Bauer, S. A. 1990. *Azolla-Anabaena* symbiosis: Use in agriculture. *In* A.N. Rai (Ed.). CRC handbook of symbiotic cyanobacteria, pp. 119–136. CRC Press, Inc., Boca Raton, Florida.

Ortloff, C. R., and A. L. Kolata. 1996. Tiwanaku raised-field agriculture in the Lake Titicaca basin of Boliva. *In* A. Kolata, (Ed.). Tiwanaku and its hinterland: Archaeology and paleoecology of an Andean civilization. 1. Agroecology, pp. 109–152. Smithsonian Institution Press, Washington, DC.

Raynal-Roques, A. 1992. Macrophytes: The higher plants. VI. 2a. 1. *In* C. Dejoux and A. Iltis (Eds.). Lake Titicaca: A synthesis of limnological knowledge, pp. 223–231. Kluwer Academic Publishers, Dordrecht, the Netherlands.

Reynaud, P. A. 1982. The use of *Azolla* in West Africa. *In* P. H. Graham and S. C. Harris. (Eds.). Biological nitrogen fixation technology for tropical agriculture, pp. 565–578. Centro International de Agricultura Tropical, Cali, Colombia.

Roger, P. A., and S. A. Kulasooriya. 1980. Blue-green algae and rice, pp. 1–112. The International Rice Research Institute, Los Banos, the Philippines.

Singh, P. K. 1979. Use of *Azolla* rice production in India. *In* Nitrogen and rice, p. 407. International Rice Research Institute, Los Banos, the Philippines.

Tjepkema, J. D., and H. J. Evans. 1976. Nitrogen fixation associated with *Juncus balticus* and other plants of Oregon wetlands. Soil Biol. Biochem. 8: 505–509.

Turner, G. L., and A. H. Gibson. 1980. Measurement of nitrogen fixation by indirect means. *In* F. J. Bergersen. (Ed.). Methods for evaluating biological nitrogen fixation, pp. 111–138. John Wiley & Sons, Ltd., New York, New York.

Ventura, W., and I.Watanabe. 1983. N-15 dilution technique of assessing the contribution of nitrogen fixation to rice plants. Soil Sci. Plant Nut. 29: 123–133.

Vincent, W. F., C. L. Vincent, M. T. Downes, and P. J. Richerson. 1985. Nitrate cycling in Lake Titicaca (Peru-Bolivia): The effects of high altitude and tropicality. Freshw. Biol. 15: 31–42.

———, W. Wurtsbaugh, C. L. Vincent and P. J. Richerson. 1984. Seasonal dynamics of nutrient limitation in a tropical high-altitude lake (Lake Titicaca, Peru–Bolivia): Application of physiological bioassays. Limnol. Oceanogr. 29: 540–552.

Watanabe, I. 1982. *Azolla-Anaebaena* symbiosis—its physiology and use in tropical agriculture. *In* Y. R. Dommergues and H. G. Diem (Eds.). Microbiology of tropical soils and plant productivity, p. 169. Martinus Nijhoff, the Hague, the Netherlands.

WURTSBAUGH, W., W. F. VINCENT, R. ALFARO, AND C. L. VINCENT. 1984. Nitrogen limitations in a tropical alpine lake, Lake Titicaca (Peru/Bolivia). Verh. Intern. Verein. Limnol. 22: 1252 (abs).

———, W. F. VINCENT, R. ALFARO, C. L. VINCENT, AND P. J. RICHERSON. 1985. Nutrient limitations of algal growth and nitrogen fixation in a tropical alpine lake, Lake Titicaca (Peru/Bolivia). Freshw. Biol. 15: 185–195.

YE, LI SHI. 1984. *Azolla* in the paddy fields of eastern China. *In* Organic matter and rice, pp. 169–178. The International Rice Institute, Los Banos, the Philippines.