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RESEARCH PAPER

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Variations in the microalgal structure in paddy soil in Osaka, Japan: comparison between surface and subsurface soils

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Abstract Seasonal variations in microalgal communities were compared between surface and subsurface paddy soils in Osaka, Japan. Soil samples were collected from depths of 0–1 (surface), 8–9, and 17–18 cm. Diatom cells were counted directly, and the numbers of other microalgae were estimated using a culture method. The microalgal community as well as the soil properties changed drastically in the surface soil as a consequence of alternate flooding and drainage. In the soil collected at a depth of 0–1 cm, the cell density of diatoms and the viable count of other microalgae markedly increased, and *Chlorella* spp., *Nitzschia* spp., and *Navicula* spp. were predominant during the flooding period, whereas *Scenedesmus* spp. and *Hantzschia* spp. were predominant during the drainage period. In contrast, in the soils collected at depths of 8–9 and 17–18 cm, the cell density of diatoms and the viable count of other microalgae remained constant. Despite the unavailability of light, a large number of microalgae were present in these subsurface soils throughout the annual cultivation cycle, and *Scenedesmus* spp. and *Nitzschia* spp. were always dominant. Cyanophytes were also present at all the depths but had low relative frequencies. These results suggest that the algae that are predominant in paddy soil can survive not only drastic changes in water content but also complete darkness.

Key words Seasonal variation · Microalgae · Surface paddy soil · Subsurface paddy soil

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Introduction

Paddy fields are artificial wetlands that are frequently disturbed by repetitive intense agricultural practices such as flooding, drainage, and ploughing as well as the application of fertilizers and herbicides. Algae function as primary producers in paddy water and surface soil and occupy an important position in the paddy field ecosystem during both the flooding and drainage periods (Roger 1996). Cyanophytes are known to have beneficial effects on the productivity of wetland rice fields (Mandal et al. 1999). In Thailand and Laos, filamentous algae such as *Spirogyra* spp. in paddy fields and vicinal waters are occasionally used for food (according to interviews with villagers; Peerapornpisal 2005). On the other hand, in Japanese and Australian paddy fields, large mats and slime that are dominated by filamentous algae or diatoms retard the growth of rice seedlings (Yamagishi and Hashizume 1974; Noble and Happey-Wood 1987). This problem has resulted in the use of herbicides and algicides.

Many species of cyanophytes have been reported to be capable of fixing atmospheric N_2. Additionally, many studies have been conducted in Asian countries to evaluate the ability of cyanophytes to enhance crop yield when they are added to paddy soils (Roger and Kulasooriya 1980; Reddy and Roger 1988; Watanabe and Liu 1992). In Japan, some ecological studies demonstrated that microalgal cell densities in paddy water decrease because of grazing or the lack of available light resulting from the rice canopy (Ichimura 1954; Kurasawa 1957; Kikuchi et al. 1975; Taira and Hogetsu 1987). The algal community structures in paddy water or paddy soil differ depending on the conditions of fertilization (Taira and Hogetsu 1987; Fujita and Nakahara 1999). Diatoms are well known to be abundant in paddy fields (Kikuchi et al. 1975; Taira and Hogetsu 1987; Ohtsuka and Fujita 2001). However, regardless of the algal group, limited information is available regarding algal composition or succession in paddy soils.

It is generally assumed that algae, being phototrophic microorganisms, are restricted to illuminated areas. How-

ever, common cultivation practices, such as ploughing and puddling, carry the algae in the paddy water and surface soil to the deeper unilluminated areas and those in the deeper soil to the illuminated areas. Soil algae are known to survive below the soil surface, and some strains can grow heterotrophically (Khoja and Whitton 1971; Metting 1980). Viable algal propagules are present at depths as great as 1 m (Kamat and Patel 1973). Thus, the algal population may survive in subsurface paddy soils despite the dark conditions.

In the present study, we investigated the seasonal variations in the abundance and composition of microalgae in paddy soil, focusing on the differences between the surface and subsurface layers.

Materials and methods

Study site

Sampling was conducted in the paddy field of the experimental farm of Kyoto University, which is located near the center of Takatsuki in Osaka Prefecture in central Japan, from June 11, 1995 to April 26, 1996 (Fig. 1). The area of the experimental paddy field is 0.1 ha. The cultivation calendar of the paddy field is shown in Table 1. The paddy soil was ploughed to a depth of approximately 15 cm. The irrigation water for the paddy field was obtained from an agricultural reservoir approximately 1 km away from the farm; it flowed through a residential area including farmlands. Domestic waste may partly flow into the irrigation ditch. In this article, flooding indicates that the paddy field was submerged in irrigated paddy water, and drainage indicates that the paddy water was drained and that no irrigation water flowed into the paddy field. Flooding was started on June 12, 1995. The depth of the water in the paddy field was

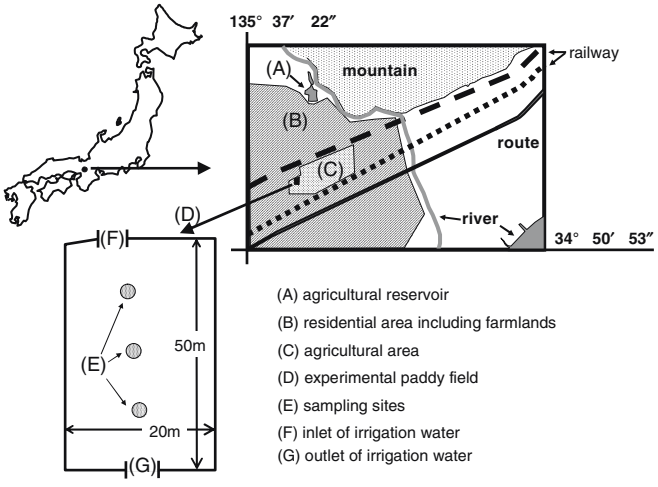


Fig. 1. Map of the experimental paddy field and the sampling sites

Table 1. Cultivation calendar of the paddy field

Year	Month	Date	Sampling	Agricultural practice	Fertilizer, pesticide
1995	April	21		Ploughing	
	May	25		Ploughing	
	June	11	○[a]		
		12		Flooding	
		13	○	Fertilization	30-100-30 kg ha^{-1}[c]
				Puddling and leveling	
		14	○		
		15	○		
		16	○	Transplanting	
		17	○		
		18	○		
		29		Herbicide application	Bensulfuron methyl: esprocarb
	July	13		Topdressing	20-0-20 kg ha^{-1}
		18	○		
	August	2		Topdressing	20-0-20 kg ha^{-1}
		14		Topdressing	30-0-30 kg ha^{-1}
		18	○		
	September	1		Insecticide application	Uprofezin: fenobucarb
	October	6	○	Beginning of drainage	
		9	○	(Disappearance of paddy water)	
		10	△[b]		
		11	○		
		12	△		
		16	○		
		20		Harvest	
		23		Ploughing	
	December	28	○		
1996	January	12		Ploughing	
	March	11	○		
	April	26	○		

[a] Soil properties were determined and microalgae were counted
[b] Soil properties were determined but microalgae were not counted
[c] N-P-K (chemical fertilizer)

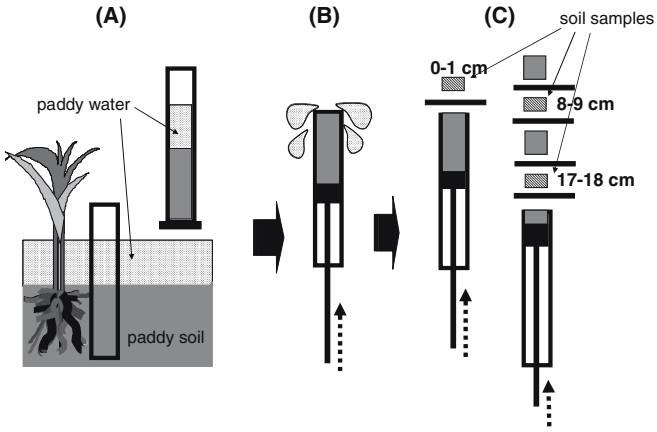


Fig. 2. Method for collecting soil samples

maintained at 5–10 cm during the flooding period. After sampling on October 6, 1995, the paddy water was gradually drained, and the drainage was completed on October 9, 1995.

Sampling

The sampling dates are also shown in Table 1. Frequent surveys were conducted immediately after flooding and drainage because it was expected that the drastic resultant physicochemical changes would affect the algal population in the paddy soil.

Three sampling sites were selected in the field. These sites were located more than 10 m away from the water inlet and outlet points (Fig. 1E). Duplicate cores of soil were collected at each sampling site by using a plastic core sampler with a diameter of 38 mm and a length of 500 mm (Fig. 2A). During the flooding period, the surface water, which occasionally contained large algal mats close to the bottom, was removed (Fig. 2B). Soil subsamples were collected at depths of 0–1, 8–9, and 17–18 cm from each soil core as representatives of the illuminated zone, the unilluminated zone that is ploughed as a result of agricultural practices, and the unploughed unilluminated zone, respectively (Fig. 2C).

Counting of microalgae

A composite sample was prepared from six replicate subsamples collected at each depth. Each composite sample (wet weight, 0.8–1.5 g) was placed in a flask and diluted 100 fold with distilled water. After shaking for 1 h with a mechanical rotary shaker, an aliquot of the soil suspension was fixed with Lugol's solution at a final concentration of 1% for diatom counts. The number and relative frequency of diatoms were determined by directly counting the diatom frustules in the fixed soil suspension using a hemocytometer under a light microscope. The frustules with chloroplasts were counted as viable diatom cells. To estimate the number of microalgae other than diatoms, we adopted the following culture method. A 0.1-ml aliquot of the soil suspension was filtered through grid-marked filters with a pore size of 0.45 μm. These filters with the trapped soil particles and algal cells were placed on 1% agar CT medium (Watanabe and Ichimura 1977) in a petri dish and incubated at 20°C under a 14:10 L:D cycle with a photon flux density of 40 $\mu mol\, m^{-2} s^{-1}$. Incubation was started within 12 h of soil sampling. After more than 3 weeks of incubation, the microalgal colonies that had appeared on the surface of the filters were counted under a stereoscopic microscope. The microalgal counts were conducted on five replicate filters for each composite soil sample. After incubation, one of the five replicate filters was used to determine the relative frequency of the microalgae. A total of 100 colonies were isolated along the grid on the filter, and subsequently, they were cultured in Bold's basal medium (Bischoff and Bold 1963), BG11 medium (Rippka et al. 1979), or agar/liquid CT medium. These isolates were identified based on the morphological characteristics that appear during their life cycle (Komárek and Fott 1983; Anagnostidis and Komárek 1985, 1988, 1990; Komárek and Anagnostidis 1986, 1989; Ettl and Gärtner 1995).

Physicochemical parameters of paddy soil

The water content of the soil samples was determined gravimetrically by heating them at 105°C for 24 h. The pH of the soil samples was determined from soil suspensions that were prepared by mixing air-dried soil and water in a ratio of 1:5 (TOA HM-5S). The total carbon (TC) and total nitrogen (TN) contents were determined using a CN analyzer (Yanaco MT-500). The phosphorus content was determined by the Bray No. 2 method (Bray-P) as follows (Blakemore et al. 1987). The phosphorus in the soils was extracted by shaking 2.5 g air-dried soil in 25 ml 0.01 M HCl and 0.03 M NH_4F. The extracted phosphorus was estimated by the molybdate blue method using ascorbic acid as the reductant.

Results

Soil properties

Figure 3 shows the seasonal variations in the water, TC, TN, Bray-P content, and pH in the soils. The water content of the soil samples collected at a depth of 0–1 cm (0–1 cm soil) increased sharply immediately after flooding, whereas it decreased gradually after drainage. On the other hand, the water content of the soil samples collected at depths of 8–9 (8–9 cm soil) and 17–18 cm (17–18 cm soil) remained in the range of 20.0%–30.0% throughout the experimental period. The TC and TN content in the 0–1 cm soil also increased considerably during the flooding period, although there were marginal changes in the 8–9 cm and 17–18 cm soils. There were few differences in seasonal variation in pH and Bray-P between the 0–1 cm soil and those collected at subsurface depths. The mean TC, TN, and Bray-P content were the highest in the 0–1 cm soil.

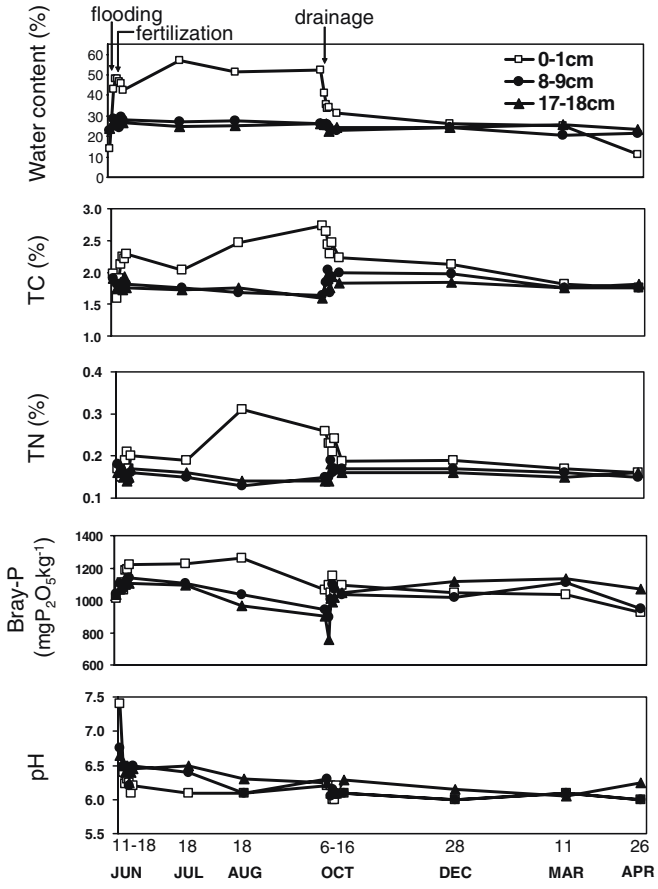


Fig. 3. Seasonal variations in water, total carbon (TC), total nitrogen (TN), Bray-P content, and pH

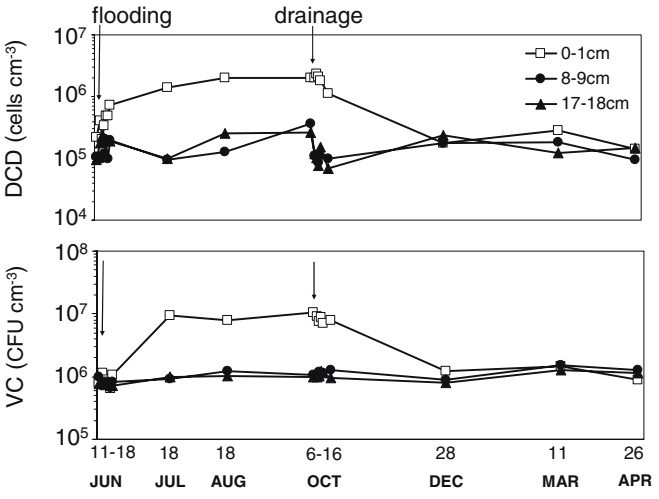


Fig. 4. Seasonal variations in total cell density of diatoms (*DCD*) and total viable count of other microalgae (*VC*)

Table 2. Mean relative frequency of each diatom genus (%)

Genus	Depth of soil		
	0–1 cm	8–9 cm	17–18 cm
Nitzschia	39.0	15.6	16.6
Navicula	15.1	21.5	23.6
Hantzschia	8.6	10.8	13.7
Caloneis	1.6	1.2	1.4
Craticula	<1.0	1.4	<1.0
Encyonema	<1.0	1.1	<1.0
Fallacia	2.3	1.8	<1.0
Luticola	7.5	2.3	1.6
Neidium	1.1	2.6	2.2
Pinnularia	11.4	7.7	6.4
Placoneis	1.5	5.2	4.8
Sellaphora	<1.0	<1.0	<1.0
Stauroneis	1.6	1.0	<1.0
Surirella	4.0	3.8	1.7
Tryblionella	1.9	7.1	8.0
Others	3.0	16.2	16.8

Total viable counts of microalgae

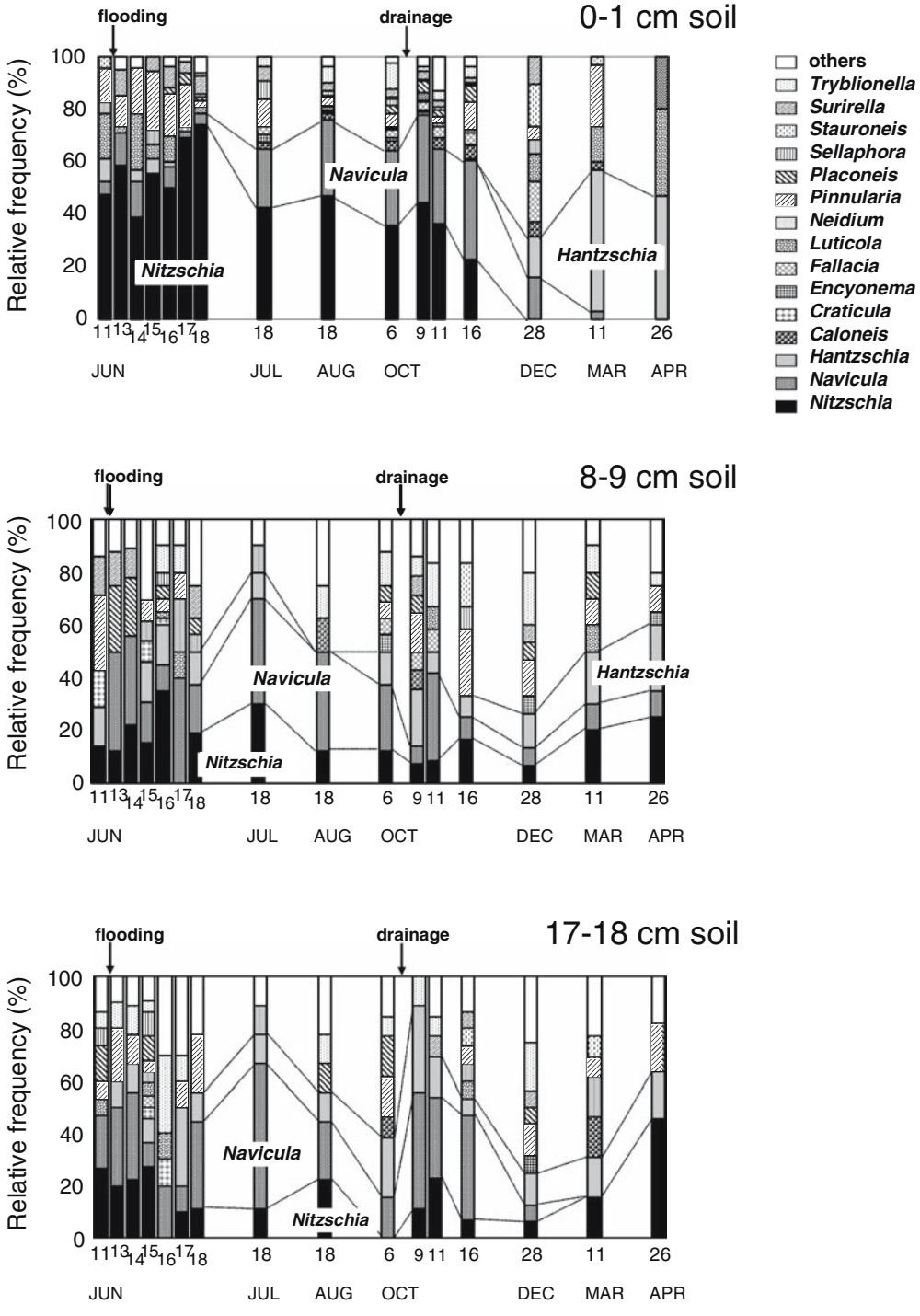
In the present study, we have used the term DCD to indicate the total cell density of the viable diatom cells and VC to indicate the total viable count of the other microalgae obtained by counting the colonies on the filters on the agar CT medium after incubation.

Figure 4 shows the seasonal variations in the DCD and the VC. The DCD in the 0–1 cm soil gradually increased immediately after flooding, was maintained at 10^6 cells cm^{-3} during the flooding period, and decreased after drainage. In contrast, DCD in the 8–9 cm and 17–18 cm soils changed slightly throughout the experimental period, and the levels were similar to that observed in the 0–1 cm soil during the drainage period.

No significant increase was observed in the VC of the 0–1 cm soil 1 week after flooding, although the water content increased rapidly from 13.9% to more than 40%. VC in the 0–1 cm soil reached approximately 10^7 colony-forming units (CFU) cm^{-3} on July 18 and remained high until October 16, i.e., at 10 days after the start of drainage, and thereafter decreased to a level similar to that before flooding. VC in the 8–9 cm and 17–18 cm soils remained constant throughout the experimental period.

Seasonal variations in the generic composition of microalgae

The generic composition and mean relative frequencies of diatoms are shown in Fig. 5 and Table 2. Each relative frequency indicates proportion compared to DCD. With regard to diatoms in the 0–1 cm soil, we cite the published data from a study (Ohtsuka and Fujita 2001) that discussed the seasonal pattern and the taxonomic characteristics of diatom species by using the same soil samples. The seasonal variation in the generic composition of diatoms in the 0–1 cm soil was different from those at the subsurface depths. In the 0–1 cm soil, *Nitzschia* spp. continued to be dominant, with high relative frequencies ranging from 23% to 69% from June 11 before flooding to October 16 just after drainage; however, these diatoms were not observed during the drainage period. The relative frequency of *Navicula* spp. increased during the flooding period and decreased after



drainage. The relative frequency of *Hantzschia* spp. showed a marked increase after drainage, with a maximum relative frequency of 53% on March 11, although it was always very low during the flooding period. The relative frequency of *Luticola* spp. was rather high during the drainage period and 1 week after flooding. *Caloneis* spp., *Fallacia* spp., *Placoneis* spp., and *Tryblionella* spp. appeared after flooding and disappeared with the decrease in water content after drainage. *Pinnularia* spp., especially *Pinnularia subcapitata* W. Greg. var. *elliptica* Krasske, were observed throughout the experimental period.

In the 8–9 cm and 17–18 cm soils, the relative frequency of *Navicula* spp. remained high during the flooding period but decreased after drainage. The high relative frequency of *Nitzschia* spp. was maintained in these subsurface soils during the experimental period. *Hantzschia* spp., *Pinnularia* spp., *Placoneis* spp., and *Tryblionella* spp. occasionally showed high relative frequencies both in the 8–9 cm and

Fig. 5. Seasonal variations in generic composition of diatoms

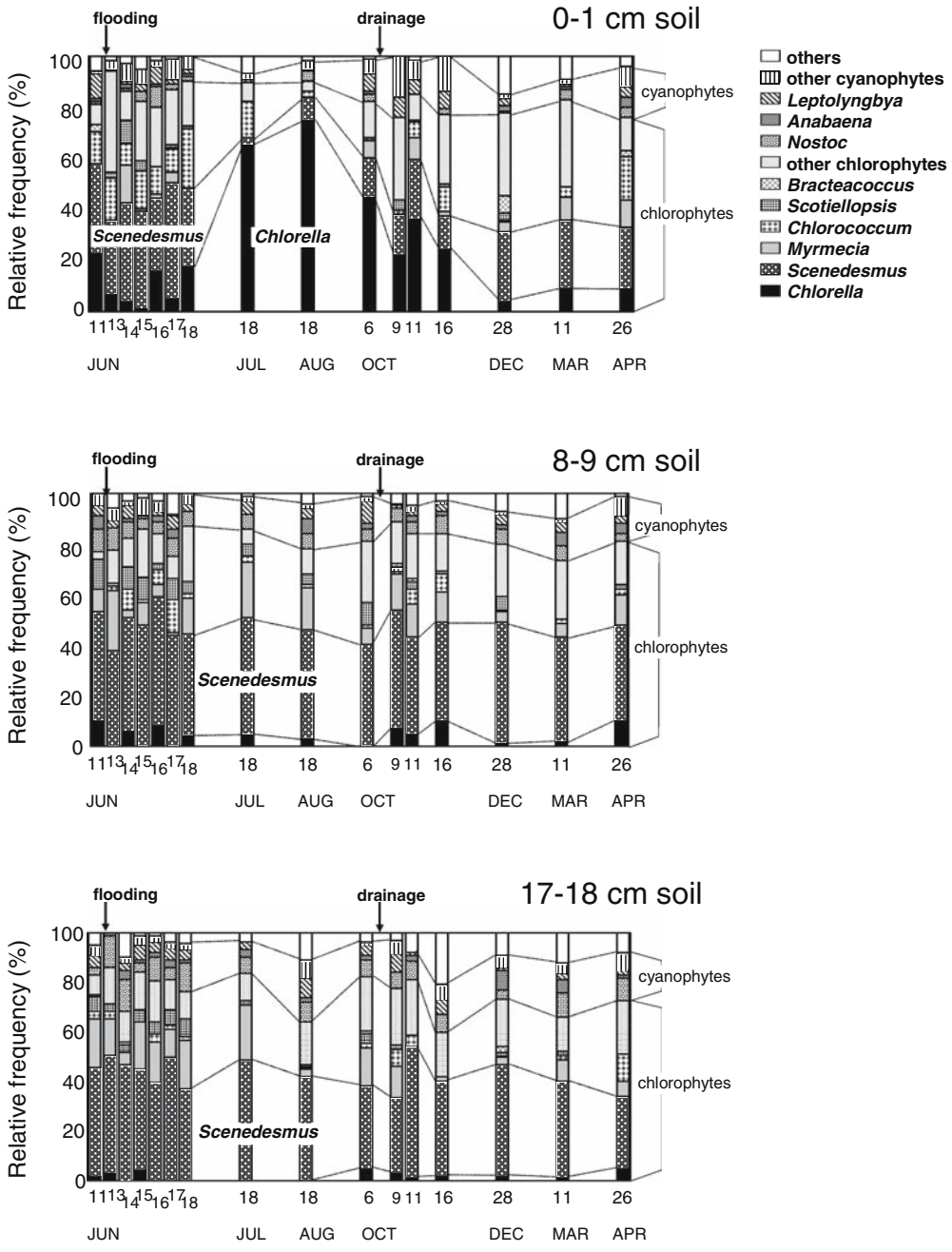


Fig. 6. Seasonal variations in generic composition of microalgae excluding diatoms

17–18 cm soils. However, no specific trends in their variations were observed.

The generic composition and mean relative frequencies of the microalgae, except diatoms, are shown in Fig. 6 and Table 3. Each relative frequency indicates proportion relative to VC.

A variety of chlorophytes were observed at every depth. In the 0–1 cm soil, *Scenedesmus* spp., *Chlorococcum* spp., and *Chlorella* spp. were predominant, showing high relative frequencies during the drainage period and 1 week after flooding. *Scenedesmus* spp. included two abundant species (Fig. 7A–C) and other infrequent species. *Chlorella* spp. included *Chlorella vulgaris* Beij., *Chlorella sorokiniana* Shihira et Krauss, and several other species. *Chlamydomonas* spp. showed low relative frequencies (<6%) and were present only in the 0–1 cm soil for several days after flooding, during which time they bloomed in the surface paddy water (data not shown). During the flooding period, *Chlorella* spp. were predominant, showing a maximum relative frequency of 75% on August 18. The most predominant species among *Chlorella* spp. in the flooded 0–1 cm soil (Fig. 7D) were rarely observed in the drained 0–1 cm soil as well as the subsurface soils. Among cyanophytes, *Nostoc* spp. and *Leptolyngbya* spp. were commonly observed.

In the 8–9 cm and 17–18 cm soils, *Scenedesmus* spp. continued to be dominant regardless of the season or agricultural practices, and their relative frequencies were greater than those obtained from the drained 0–1 cm soil. The

Table 3. Mean relative frequencies of microalgal genera excluding diatoms (%)

Alga genus	Depth of soil		
	0–1 cm	8–9 cm	17–18 cm
Chlorophytes	83.7	81.5	76.2
Chlorella	22.7	4.4	1.7
Scenedesmus	25.2	43.1	41.6
Myrmecia	3.9	10.7	11.1
Chlorococcum	9.5	3.4	2.4
Scotiellopsis	1.6	4.8	3.0
Bracteacoccus	<1.0	<1.0	<1.0
Other chlorophytes	20.0	14.9	16.0
Cyanophytes	12.7	14.8	17.3
Nostoc	2.2	6.0	7.8
Anabaena	<1.0	2.3	2.2
Leptolyngbya	3.9	3.6	3.8
Other cyanophytes	5.7	2.9	3.4
Others	3.6	3.6	6.5

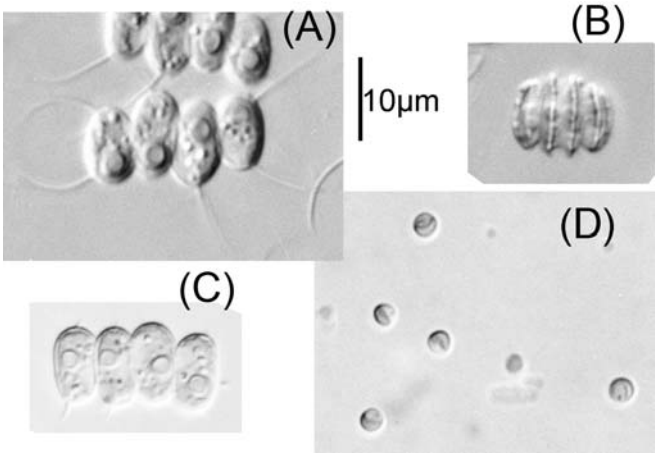


Fig. 7. Two dominant species of *Scenedesmus* (**A, B, C**) and dominant species of *Chlorella* (**D**). **A** and **B** are different stages of the same species. Such morphological variability in the culture was observed in *Scenedesmus* isolated from a Connecticut soil (Trainor 1964, 1965, 1969)

relative frequencies of the two abundant species of *Scenedesmus* averaged 13.1% and 15.5%. Among chlorophytes, *Myrmecia* spp. sometimes showed high relative frequencies, and *Scotiellopsis terrestris* (Reisigl) Punčoch. et Kalina frequently occurred with low relative frequencies at both 8–9 cm and 17–18 cm. *Nostoc* spp. were the most predominant cyanophytes in both the 8–9 cm and 17–18 cm soils, followed by *Leptolyngbya* spp. and *Anabaena* spp. All the microalgal species isolated from the 8–9 cm and 17–18 cm soils were observed in the 0–1 cm soil.

The relative frequencies of other algae, including *Heterococcus* sp. and *Xanthonema* sp., were always considerably low at each depth. Algal species belonging to Charophyceae and Ulvophyceae were observed only occasionally.

Discussion

The results of the present study can be summarized based on the following three points. (1) During the experimental period, the community structure of the microalgae in the paddy soil changed markedly in the surface soil but only marginally in the deeper soil. (2) High microalgal abundance was continuously maintained in the deeper soil despite the unavailability of light. (3) *Scenedesmus* spp., *Nitzschia* spp., and *Navicula* spp. were predominant at all depths.

Based on the soil properties analyzed, it is obvious that the microalgal community in the surface soil reacted to changes in the environmental conditions, which were strongly affected by alternate drainage and flooding during rice cultivation. There are two possible reasons for the high DCD and VC during the flooding period in the 0–1 cm soil. One is influx of microalgae present in the irrigation water. The most abundant *Chlorella* species in the flooded 0–1 cm soil were rarely observed after drainage. Certain diatoms, such as *Navicula rostellata* Kütz., *Tryblionella parvula* (W. Sm.) Ohtsuka et Y. Fujita., and *Tryblionella calida* (Grunow) D.G. Mann., were present at relatively high cell densities only during the flooding period (Ohtsuka and Fujita 2001). These results imply that the *Chlorella* species and these diatoms are among aquatic species that are susceptible to desiccation and that these species flowed into the paddy field along with the irrigation water, increased in number on the soil surface, and disappeared after drainage. Another reason for the high DCD and VC is that flooding is advantageous to certain edaphic microalgae that are present throughout the cultivation cycle. The calculated VC of *Scenedesmus* spp. increased from 10^5 CFU cm^{-3} to 10^6 CFU cm^{-3} during the flooding period. Additionally, the numbers of many diatom species increase during the flooding period (Ohtsuka and Fujita 2001).

Several days after flooding, VC and the generic composition in the 0–1 cm soil rarely changed, although *Chlamydomonas* spp. bloomed in the surface paddy water; this is primarily because the dispersion of clay particles by puddling, leveling, transplanting, and intermittent rain decreased the available light that reached the soil surface. Evans (1959) indicated that the longer the resting period during drought, the longer it takes for the resting algal cells to readjust their metabolism and begin growing when water becomes available. In the present study, certain algae may have taken more than 1 week until they began growing after the drainage period, which lasted several months.

The DCD, VC, and the relative frequencies of the dominant genera in the 0–1 cm soil continued to remain high 10 days after drainage, while the water content rapidly decreased. This result indicates that the microalgae dominant during the flooding period were tolerant to such changes in the water content. Thus, the changes of water content do not necessarily induce immediate active growth or decay of microalgae.

The sum of the DCD and VC ranged from 1.0×10^5 to 1.3×10^7 CFU or cells cm^{-3} in the 0–1 cm soil. This range is

within the microalgal abundance range of 1.0×10^4 to 5.3×10^7 CFU cm^{-2} that was observed in the 0–1 cm soils of rice fields in Asian countries (Roger et al. 1987).

The constant DCD and VC in the 8–9 cm and 17–18 cm soils were equivalent to those observed in the 0–1 cm soil during the drainage period. However, a few studies on dry fields concluded that as a consequence of the availability of light soil algae were distributed abundantly in the surface soil and sparsely in the deeper soil (Muralikrishna et al. 1985; Lukešová 1993). This difference can be partially explained on the basis of the characteristics of the cultivation practices in paddy fields. Our experimental field was ploughed several times at short intervals during the cultivation cycle. Ploughing, as pointed out by Roger (1996), enables the penetration of superficial viable algae into deeper soil. Because the depth of 17–18 cm is a few centimeters deeper than the depth to which the soil was ploughed, the algae may have been carried into this layer primarily by the percolation of paddy water and rainwater (Tchan and Whitehouse 1953) and the continuous activities of root and soil fauna. Evans (1959) studied the survival of freshwater algae in pond margin litter and mud during the dry period and suggested that some algae, particularly diatoms, descended progressively into the deep layers as the water level dropped when drying occurred at the surface. This finding may help to explain the high DCD in deeper soils. The fact that the number of species decreased with an increase in the soil depth has already been demonstrated in studies conducted in various types of fields (Azevedo 1991; Lukešová 1993).

The survival mechanisms of algae in darkness are important to explain the high microalgal abundance and the predominance of specific algae in deeper soils. Many algae can resist adverse conditions, such as complete darkness, by entering into a resting stage (Anderson 1976). Dehning and Tilzer (1989) reported that *Scenedesmus acuminatus* could survive during a prolonged period of darkness by reducing its catabolic reactions. Moreover, some soil chlorophytes and cyanophytes, including one isolate from rice fields, are known to grow by using organic compounds under dark and anaerobic conditions (Groover and Bold 1969; Metting 1980; Prosperi et al. 1992). The results of this study show that a large number of microalgae survive under dark conditions in subsurface soil. However, these results do not indicate whether the predominant algae in the 8–9 cm and 17–18 cm soils were actively growing or resting.

The culture method is useful for accurate identification of algae on the basis of observation of their life cycles. However, certain species may not grow in the media that were used for this study because of the strain selection caused by the media. In the present study, the VC of these filamentous algae appeared to be underestimated because large mats that formed by filamentous algae were not separated by shaking.

The increases in TC, TN, and Bray-P content in the 0–1 cm soil were largely caused by fertilizer applications in June, July, and August. Based on the data analyzed by the Agricultural, Food and Environmental Sciences Research Center of Osaka Prefecture, the TC and TN content and the pH in paddy soils in Osaka Prefecture are 1.76% ± 0.40% (mean ± SD; $n = 83$), 0.16% ± 0.03%, and 5.9 ± 0.5, respectively. The mean Bray-P content of 84 Japanese paddy soils was 129 mg P_2O_5 kg^{-1} (Kyuma 2004) and that of three paddy soils in Kyoto Prefecture was 356 ± 50 mg P_2O_5 kg^{-1} (Fujita and Nakahara 1999). Compared with these values, the Bray-P content determined in this study was remarkably high, although the TN and TC content and pH were similar.

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References

Anagnostidis K, Komárek J (1985) Modern approach to the classification system of cyanophytes. 1: Introduction. Arch Hydrobiol Suppl 71 (Algol Stud 38–39):291–302

Anagnostidis K, Komárek J (1988) Modern approach to the classification system of cyanophytes. 3. Oscillatoriales. Arch Hydrobiol Suppl 80 (Algol Stud 50–53):327–472

Anagnostidis K, Komárek J (1990) Modern approach to the classification system of cyanophytes. 5. Stigonematales. Arch Hydrobiol Suppl 86 (Algol Stud 59):1–73

Anderson OR (1976) Respiration and photosynthesis during resting cell formation in *Amphora coffeaeformis* (Ag.) Kütz. Limnol Oceanogr 21:452–456

Azevedo MTP (1991) Edaphic blue-green algae from the São Paulo Botanical Garden, Brazil. Algol Stud 64:503–526

Bischoff HW, Bold HC (1963) Phycological studies. IV. Some soil algae from Enchanted Rock and related algal species. Publication 6318. University of Texas, Austin

Blakemore LC, Searle PL, Daly BK (1987) Methods for chemical analysis of soils. Scientific Report 80. New Zealand Soil Bureau, Auckland

Dehning I, Tilzer MM (1989) Survival of *Scenedesmus acuminatus* (Chlorophyceae) in darkness. J Phycol 25:509–515

Ettl H, Gärtner G (1995) Syllabus der Boden-, Luft- und Flechtenalgen. Fischer, Stuttgart

Evans JH (1959) The survival of freshwater algae during dry periods. Part II. Drying experiments. III. Stratification of algae in pond margin litter and mud. J Ecol 47:55–81

Fujita Y, Nakahara H (1999) Effects of cultivation conditions on algal communities in paddy soils (in Japanese with English abstract). Jpn J Limnol 60:77–86

Groover RD, Bold HC (1969) Phycological studies. VIII. The taxonomy and comparative physiology of the Chlorosarcinales and certain other edaphic algae. Publication 6907. University of Texas, Austin

Ichimura S (1954) Ecological studies on the plankton in paddy fields. I. Seasonal fluctuations in the standing crop and productivity of plankton. Jpn J Bot 14:267–279

Kamat ND, Patel MZ (1973) Soil algae of a rice field at different depths. Botanique 4:101–106

Khoja T, Whitton BA (1971) Heterotrophic growth of blue-green algae. Arch Microbiol 79:280–282

Kikuchi E, Furusaka C, Kurihara Y (1975) Surveys of the fauna and flora in the water and soil of paddy fields. Rep Inst Agric Res Tohoku Univ 26:25–35

Komárek J, Anagnostidis K (1986) Modern approach to the classification system of cyanophytes. 2. Chroococales. Arch Hydrobiol Suppl 73 (Algol Stud 43):157–226

Komárek J, Anagnostidis K (1989) Modern approach to the classification system of cyanophtes. 4. Nostocales. Arch Hydrobiol Suppl 8 (Algol Stud 56):247–345

Komárek J, Fott B (1983) Chlorophyceae (Grünalgen), Ordnung Chlorococcales. In: Huber-Pestalozzi G (ed) Das Phytoplankton des Süsswassers. Systematik und Biologie. 7. Teil 1. Hälfe. (Die Binnengewässer, Band XVI, in German.) E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, pp 1–1044

Kurasawa H (1957) The phytoplankton zooplankton relationships in two paddy fields in central Japan. J Fac Sci Hokkaido Univ Ser IV Zool 13:180–186

Kyuma K (2004) Paddy soil science. Kyoto University Press, Kyoto

Lukešová A (1993) Soil algae in four secondary successional stages on abandoned fields. Algol Stud 71:81–102

Mandal B, Vlek PLG, Mandal LN (1999) Beneficial effects of blue-green algae and *Azolla*, excluding supplying nitrogen, on wetland rice fields: a review. Biol Fertil Soils 28:329–342

Metting B (1980) New species of green microalgae (Chlorophyta) from an eastern Washington silt loam. Phycologia 19:296–306

Muralikrishna PVG, Megharaj M, Venkateswarlu K (1985) Occurrence of soil algae as influenced by profile depth and amendments. Phykos 24:42–45

Noble JC, Happey-Wood CM (1987) Some aspects of the ecology of algal communities in ricefields and rice irrigation systems of southern New South Wales. J Aust Inst Agric Sci 53:170–184

Ohtsuka T, Fujita Y (2001) The diatom flora and its seasonal changes in a paddy field in central Japan. Nova Hedwigia 73:97–128

Peerapornpisal Y (2005) Freshwater algae in northern Thailand (in Thai and English). Biodiversity Research and Training Program, Bangkok

Prosperi C, Boluda L, Luna C, Fernandez-Valiente E (1992) Environmental factors affecting in vitro nitrogenase activity of cyanobacteria isolated from rice-fields. J Appl Phycol 4:197–204

Reddy PM, Roger PA (1988) Dynamics of algal populations and acetylene-reduction activity in five rice soils inoculated with blue-green algae. Biol Fertil Soils 6:14–21

Rippka R, Deruelles B, Waterbury JB, Hardman M, Stanier Y (1979) Generic assignments. Strain histories and properties of pure culture of Cyanobacteria. J Gen Microbiol 111:1–61

Roger PA (1996) Biology and management of the floodwater ecosystem in ricefields. International Rice Research Institute, Los Baños, Philippines

Roger PA, Kulasooriya SA (1980) Blue-green algae and rice. International Rice Research Institute, Los Baños, Philippines

Roger PA, Santiago-Ardales S, Reddy PM, Watanabe I (1987) The abundance of heterocystous blue-green algae in rice soils and inocula used for application in rice fields. Biol Fertil Soils 5:98–105

Taira M, Hogetsu K (1987) Species composition of phyto- and zooplankton communities in fertilized and non-fertilized paddy fields (in Japanese with English abstract). Jpn J Limnol 48:77–83

Tchan YT, Whitehouse JA (1953) Study of soil algae. II. The variation of the soil algal population in sandy soils. Proc Linn Soc N S W 78:160–170

Trainor FR (1964) Spine distribution in several *Scenedesmus* cultures. Am J Bot 51:995–1001

Trainor FR (1965) A study of unialgal cultures of *Scenedesmus* incubated in nature and in the laboratory. Can J Bot 43:701–706

Trainor FR (1969) *Scenedesmus* morphogenesis. Trace elements and spine formation. J Phycol 5:185–190

Watanabe I, Liu CC (1992) Improving nitrogen-fixing systems and integrating them into sustainable rice farming. Plant Soil 141:57–67

Watanabe MM, Ichimura T (1977) Fresh- and salt-water forms of *Spirulina platensis* in axenic cultures. Bull Jpn Soc Phycol 25(suppl):371–377

Yamagishi A, Hashizume A (1974) Ecology of green algae in paddy fields and their control (in Japanese with English abstract). Weed Res 18:39–43