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Special Review

Original Paper

Genetic differentiation, effective population size and gene flow in marine fishes: implications for stock management

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Abstract

Many commercially exploited marine fish and mollusc species exhibit no or a low degree of genetic differentiation in neutral marker genes. This lack of genetic differentiation, typically attributed to high degree of gene flow in marine environments, has sometimes supported the thinking that genetically indistinguishable stocks can be managed as being one panmictic population. Recent comparative studies of neutral marker gene and quantitative trait differentiation in a wide variety of taxa - including several marine organisms - show that a high degree of genetic differentiation (as measured by Q_{ST}) in ecologically and economically important traits is a common place occurrence, even when the degree of differentiation in neutral marker genes (as measured by F_{ST}) is low or absent. In fact, among the empirical studies made so far, the outcome $Q_{ST} > F_{ST}$ is pervasive. This accords with the increasing evidence that natal homing and self-replenishment of local populations may be more common in marine habitats than previously anticipated. If so, the low degree of genetic differentiation in neutral genetic markers could be a simple consequence of the large effective population size (N_e) of many marine populations, effectively buffering them against differentiation due to genetic drift. However, genetic markers linked to parts of the genome under directional selection should readily diverge in allele frequencies especially when N_e is high. In fact, several recent studies have discovered that such loci provide a way to differentiate among stocks undifferentiated in neutral marker genes. Hence, the study of adaptive rather than neutral genetic differentiation among fish and shellfish populations might provide practical tools for stock identification and thereby contribute to improved fisheries policies.

Introduction

The world's seas and marine ecosystems are in a dire state due to human activities (e.g. Roberts, 2007). The footprint of human impact on the world's oceans is strong and widespread (Halpern *et al.*, 2008), and many marine fish populations are fully or over-exploited to the point that declines and stock collapses have occurred (Pauly *et al.*, 2002; Myers and Worm, 2003; Mullon *et al.*, 2005). According to some predictions, all current fisheries will be collapsed by the year 2048 if the exploitation continues at current rates (Worm *et al.*, 2006).

The drastic population size reductions of many fishes due to exploitation will inevitably reduce their effective population sizes (N_e), and hence, increase the risk of loss of genetic variability as observed already in some (e.g. Hauser *et al.*, 2002; Hoarau *et al.*, 2005), but not in all (Poulsen *et al.*, 2006) studied species. Loss of genetic diversity and locally adapted populations (and species) can compromise stability and recovery potential of marine ecosystems, as well as impair their ability to adapt to changing environmental conditions and thereby to their capacity to provide food for the growing human population (Worm *et al.*, 2006). Likewise, by imposing selection towards earlier maturation at smaller size, fisheries are expected to lead to genetic changes and erosion of genetic variation in exploited populations (e.g. Law, 2000; Stokes and Law, 2000; Heino and Godø, 2002; Kuparinen and Merilä, 2007). Such evolutionary changes, if occurring, could also reduce the capacity of populations to recover from over-exploitation (Walsh *et al.*, 2006; Hutchings *et al.*, 2007).

In order to maintain viable and locally adapted fish stocks, proper management policies are needed. To this end, population genetic studies of marine fish populations have assumed an important role in decision-making (e.g. Waples, 1998; Kenchington *et al*., 2003; Knutsen *et al*., 2003; see also: Seeb *et al*., 2007). Neutral genetic markers can provide valuable information about geographic structuring, gene flow and demographic history of populations, information that can be highly relevant for conservation and management purposes (e.g. Frankham *et al*., 2002; Kenchington *et al*., 2003; Maes and Volckaert, 2007). However, the information conveyed by neutral genetic markers is often, or even typically, limited in two important respects. First, if truly neutral, (i.e. unlinked to traits under selection; see below), variability in neutral genetic markers may not provide any information about genetic variability and degree of genetic differentiation in ecologically and economically important traits (Pearman, 2001; Merilä and Crnokrak, 2001; McKay and Latta, 2002; Leinonen *et al*., 2008). Second, a low degree of genetic differentiation in neutral genetic markers among many populations of marine fishes is a rule rather than an exception (reviews in: Ward *et al*., 1994; Ward, 2004; see e.g. O'Reilly *et al*., 2004; Dannewitz *et al*., 2005; Jørgensen *et al*., 2005; Florin and Höglund, 2007 for examples of recent case studies), limiting the utility of neutral marker variability in stock identification. In the following, we will focus on these two problems and highlight their implications for practical fish stock management.

1. Neutral marker gene vs. quantitative trait differentiation

Given the logistic difficulties in assessing the genetic basis of ecologically important traits in marine fish populations, estimates of genetic structuring with respect to ecologically important traits are still scarce (Swain and Foote, 1999; Conover *et al*., 2006). However, there is ample evidence for genetic determination and high degree of genetic variability for fitness-related traits such as size, growth rate and fecundity in marine populations both from aquaculture experiments (Kinghorn, 1983; Jónasson *et al*., 1997; Conover, 2000) and from experimental studies of fisheries induced evolution (e.g. Conover and Munch, 2002; Walsh *et al*., 2006). Further, in those few cases where wild marine fish populations have been tested for local adaptation, even in the absence of any obvious barriers to gene flow, evidence for genetic structuring in respect to quantitative traits has been found (Conover and Present, 1990; Conover 1998; Conover *et al*., 2006). Likewise, recent work in freshwater fishes has revealed that even in the face of high levels of gene flow, significant adaptive genetic differentiation can still take place (Saint-Laurent *et al*., 2003; Hendry and Taylor, 2004).

This evidence set aside, the most general and powerful argument against taking lack of differentiation in neutral genetic markers as evidence for lack of genetic structuring among populations comes from the comparative studies of marker and quantitative genetic differentiation (reviews in: Merilä and Crnokrak, 2001; McKay and Latta, 2002; Leinonen *et al*., 2008). In the majority of studies covering a wide variety of organisms, the differentiation in genes coding quantitative traits - as measured by the Q_{ST}-index (Spitze, 1993) exceeds that in neutral marker genes (as measured by the F_{ST}-index; Wright, 1951) in many times (Fig. 1A). What is particularly noteworthy in these data is that this difference holds true in the majority of F_{ST} vs. Q_{ST} comparisons where the level of neutral marker gene differentiation is very low ($F_{ST} \leq 0.03$; Fig. 1A) as it typically is for marine fish species (Waples, 1998; Ward, 2004). For this low level of differentiation, Waples (1998) showed that unless very large sample sizes and highly polymorphic markers are available, the paradigm of panmixia is not easily rejected due to lack of statistical power of the tests. Yet, as the data in Fig. 1 shows, substantial genetic differentiation in quantitative traits is possible even when neutral marker genes indicate no differentiation at all.

Although Q_{ST}-estimates from fish studies are still scarce, all available estimates show patterns similar to those from other taxa: Q_{ST}-estimates exceed consistently the F_{ST}-estimates (Fig. 1B). It is noteworthy that all these studies have been conducted in freshwater species, some of which occur also in marine habitats. Given that the F_{ST}-estimates are typically much higher for freshwater than marine species (Ward *et al*., 1994; DeWoody and Avise, 2000) - and for freshwater populations of species that occur both in marine and freshwater habitats (e.g. Mäkinen *et al*., 2006) - these observations hint about the possibility that the contrast between the magnitude of genetic differentiation in respect to adaptive and neutral traits in marine populations might be much more pronounced than that in freshwater populations. Some evidence for this is provided by Leinonen *et al*. (2006), who observed higher degree of quantitative

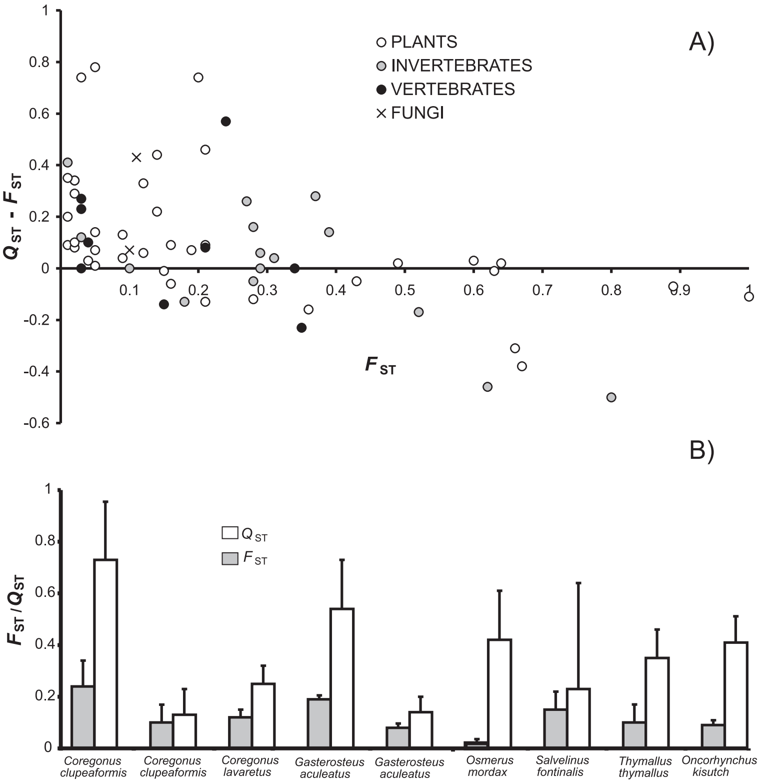

Fig. 1. Comparison of quantitative trait (Q_{ST}) and neutral marker gene (F_{ST}) differentiation in published studies. (a) Difference (= Q_{ST}-F_{ST}) divergence estimates as a function of divergence in neutral marker genes (F_{ST}) in different studies (data from Leinonen *et al.*, 2008). (b) F_{ST} and Q_{ST} estimates from published fish studies (data from: Rogers *et al.*, 2002; Koskinen *et al.*, 2002; Østbye *et al.*, 2004; Saint-Laurent *et al.*, 2004; Perry *et al.*, 2005; Leinonen *et al.*, 2006; Raeymaekers *et al.*, 2007; McClelland and Naish, 2007).

trait differentiation among marine than among freshwater populations of three-spined sticklebacks. Also, Luttikhuizen *et al.* (2003) observed a high degree of population differentiation in adaptive traits (Q_{ST} = 0.4) in a marine bivalve *Mytilus edulis* with high dispersal capacity and low degree of population structuring in neutral genetic markers (F_{ST} = 0.02). Hence, management decisions based solely on the lack of neutral genetic marker differentiation among marine fish (and shellfish) populations would probably lead to poor fisheries policies.

Before turning to proximate explanations for the low degree of neutral marker gene differentiation, we wish to provide a couple of words of warning about the limitations of Q_{ST}-estimates. First, as it can be seen from Fig. 1A, the difference between F_{ST} and Q_{ST} tends to get smaller with increasing F_{ST}. This is expected as the Q_{ST} is bounded to take values in between 0 and 1, and once F_{ST} approaches unity, the difference between the two is reduced unless Q_{ST} << F_{ST} (Hendry, 2002). Therefore, in the context of stock identification and detection of locally adapted populations, the best value for efforts invested on estimating Q_{ST} are gained from systems where F_{ST}s are known to a priori be low. Second, the estimation of Q_{ST} and its standard errors is computationally demanding, and the precision of the estimates is low unless relatively large number of populations are compared (O' Hara and Merilä, 2005; Johansson *et al.*, 2007). Apart from the logistic difficulties with quantitative genetic experiments needed to estimate Q_{ST}, both of the issues mentioned above might limit utility of Q_{ST} in the practical stock identification context. For recent meta-analyses and further discussion on the challenges in Q_{ST}-estimation, see Merilä and Cronokrak (2001) and Leinonen *et al.* (2008).

2. Gene flow or large N_e?

While low degree of genetic differentiation in neutral genetic markers among many populations of marine fishes is undisputable (e.g. Ward *et al.*, 1994; McQuinn, 1997; Ward and Elliot, 2001; Wirth and Bernatchez, 2001; Knutsen *et al.*, 2003; Nielsen *et al.*, 2003; O'Reilly *et al.*, 2004; Ward, 2004; Dannewitz *et al.*, 2005; Jørgensen *et al.*, 2005; Florin and Höglund, 2007), the causes for this shallow genetic structuring are not. The low degree of differentiation can result from many factors: high levels of gene flow, large effective population size and/or shared ancestral polymorphism attributable to recent divergence of the populations. While the third explanation may be especially relevant in recently glaciated areas, where the populations are likely to be young (Pogson *et al.*, 2001), it could also apply to more recent population turnover events (Florin and Höglund, 2007). Yet, the lack of significant population differentiation in neutral genetic markers has been traditionally ascribed to high levels of gene flow among marine populations (e.g. Ryman *et al.*, 1984; Ward et al., 1994; Waples, 1998; Ward, 2004). The lack of obvious dispersal barriers in marine environments together with often highly vagile and dispersive larval stages of marine organisms have lead to an impression of high connectivity of marine populations (Palumbi, 1992, 2003). However, this view has been changing recently (e.g. Cowen *et al.*, 2000; Levin, 2006; Metcalfe, 2006; Rocha *et al.*, 2007) and evidence for natal homing (Thorrold *et al.*, 2001; Kraus and Musick, 2001) and self-replenishment has been accumulating (Jones *et al.*, 1999; Swearer *et al.*, 1999; Cowen *et al.*, 2006; Ruzzante *et al.*, 2006). Hence, high gene flow as a general explanation for the shallow genetic structuring of marine populations is becoming tenuous.

A low degree of genetic differentiation among populations of marine species could also be attributed to large effective population sizes, which would be expected to limit differentiation due to genetic drift (e.g. Allendorf and Phelps, 1981; DeWoody and Avise, 2000). In fact, both high levels of gene flow, and large N_es in absence of gene flow are expected to leave similar signature on the genetic structure of populations. This is illustrated by the simulation results presented in Fig. 2: with N_es of several thousands, the F_{ST} will remain low even in absence of migration (Fig. 2). Hence, a low degree of genetic differentiation is expected also in the absence of gene flow if N_e is large. The reason for this is that large N_e makes genetic drift an inefficient force in altering allele frequencies over space and time, and it will take roughly N_e generations before reaching drift-migration equilibrium (e.g. Crow, 1986). Hence, allele frequencies in neutral loci in large populations are slow to change. Yet – as pointed out above - even in cases where F_{ST} is close to zero and $mN_e > 20$, divergent (e.g. dotted line in Fig. 2) selection can lead to adaptive population differentiation as has been shown for the three-spined stickleback (*Gasterosteus aculeatus*; Hendry and Taylor, 2004) and rainbow smelt (*Osmerus mordax*) populations (Saint-Laurent *et al.*, 2003).

3. N_e of marine fish populations

What do we know about the effective size of marine populations? For many commercially important species, such as Atlantic cod, the possibility of hav-

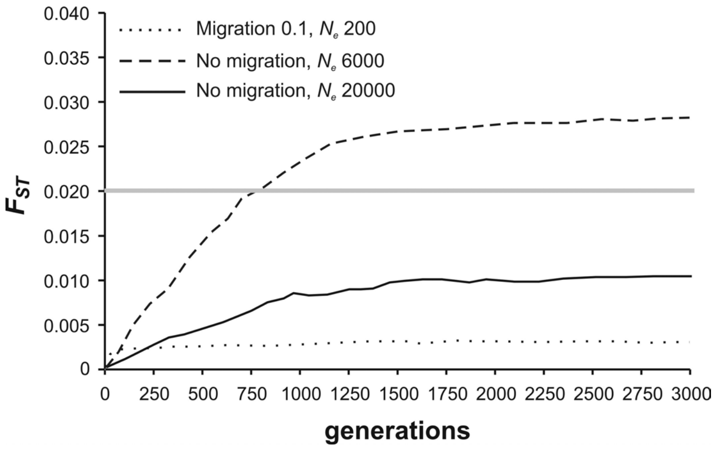

Fig. 2. A simulated example illustrating the expected influence of gene flow and effective population size on neutral marker gene differentiation (F_{ST}). Changes in F_{ST} between two populations over 3000 generations are shown, assuming different amounts of gene flow and different effective size (N_e) of populations. Each line represents an average of 100 simulations based on seven highly-polymorphic loci. Calculations assume an equal sex ratio, no mutation, random mating, free recombination and 15 possible allelic states. The grey line represents the median F_{ST} value for marine fish populations (Ward, 2004). Simulations were carried out with program EASYPOP (Balloux, 2002).

ing small N_es is quite unlikely, considering their large census population sizes and the strong fishing pressure that they have supported for centuries (Richardson and Gold, 1993; Pogson *et al*., 2001; but see Hutchinson *et al*., 2003). In fact, a recent study suggests that the effective size of local cod populations is likely to exceed several thousands of individuals (Poulsen *et al*., 2006). Estimates for other marine species are still scarce (Hauser *et al*., 2002; Turner *et al*., 2002; Árnason, 2004; Hoarau *et al*., 2005), and sometimes turned out to be surprisingly small (e.g. Turner *et al*., 2002). However, given the methodological difficulties involved in estimation of N_e with the temporal method (e.g. sampling strategy & technical artefacts, migration and cryptic population structure; see Poulsen *et al*., 2006 for detailed discussion), some caution should be exercised in attaching too much weight on individual estimates. Although we are still far from having a satisfactory picture about N_es in marine fish, the logical possibility of large N_es constraining neutral population differentiation should not be overlooked and more research would be needed on this issue.

4. Future Research needs

In our view, the main challenge for the future studies of population structuring in marine organisms is in estimating the degree of population differentiation in genes coding quantitative traits. Two basic approaches are obvious:

First, common-garden (aquaculture) experiments with fish from different origins allow estimation of the degree of additive genetic variability within and among populations and thereby Q_{ST}. Albeit a logistical challenge, this approach should be feasible even for many marine fishes (see: Conover *et al*., 2006 and their Table 1 for compilation of such experiments in marine fishes) and for molluscs in particular, because numerous species are routinely reared in aquacultures (e.g. Guo *et al*., 1999). Even in the absence of estimates of within and among population estimates of additive genetic variability for quantitative traits, some useful inference about the magnitude of Q_{ST} may still be possible if reasonable assumptions about genetic *vs*. environmental influences on phenotypic trait means can be made (e.g. Merilä, 1997; Bernatchez, 2003; Cano *et al*., 2008; Leinonen *et al*., 2008). Yet, care should be taken with the inferences as environmental effects on fish phenotypes are pervasive (e.g. Swain and Foote, 1999), and often geographically structured too (Conover, 1998; Conover *et al*., 2006).

Second, genomic approaches - such as genome scans (Schlötterer, 2003) or gene expression studies (e.g. Whitehead and Crawford, 2006; Hoffmann and Daborn, 2007) - which have recently become an area of important research (e.g. Beaumont and Balding, 2004; Larsen *et al*., 2008) provide another path for gaining information about adaptive divergence among stocks showing little differentiation in neutral genetic markers. The idea behind genome scans is that putative neutral genetic markers, such as microsatellites, will exhibit less allelic variation and more population differentiation than under neutral expectation if they are linked to selected sites (i.e. genes coding for traits of adaptive value). This phenomenon is known as hitchhiking or selective sweep (Schlötterer, 2003; Beaumont and Balding, 2004). Genome scan approaches could be expected to be of particular utility in finding signatures of selective events - and thereby also useful markers for stock-identification - in marine species because of their large effective population sizes. This is because even weak selection can overrule the effect of genetic drift in large populations (Endler, 1986) and, thereby, increase the chance of the detection of selective sweeps. Genome scan studies are still rare in marine fishes (but see: Mäkinen *et al*., 2008a,b), but hitch-hiking selection on two loci has been documented in the Atlantic cod (Nielsen *et al*., 2006; Westgaard and Fevolden, 2007). On the basis of these loci, Westgaard and Fevolden (2007) inferred that Norwegian coastal and North East Artic cods at Barents Sea form two distinct management units.

Genome scan studies can be complemented with candidate gene approaches (Coussens and Nobis, 2002) by sequencing the genomic regions next to detected selective sweeps and matching them against gene sequences identified from other species and/or available in databases such as GenBank (see: Mäkinen *et al*., 2008b for an example). In this way, it is possible to identify genomic regions containing genes coding for phenotypic traits (i.e. functional candidates) and use these in stock differentiation. From a stock management perspective, genome scan approaches should be prioritised at local scales, rather than involving populations across large geographical distances, since is at the microgeographical level where neutral markers usually fail to detect significant population structure (Lundy *et al*., 1999; Knutsen *et al*., 2003; Nielsen *et al*., 2004).

Finally, more information about effective population sizes and actual migration rates are obviously needed to test the hypothesis about the buffering effect of large N_e on population differentiation in marine fishes. Studies such as those by Hauser *et al*. (2002), Turner *et al*. (2002), Árnason (2004) and Nielsen *et al*. (2006) based on the observed changes of marker allele frequencies over time (i.e. temporal approach, Krimbas and Tsakas, 1971) show the way. However, as temporal methods estimate N_e according to the rate of allele frequencies change, migration can cause a population to behave as if strong drift was changing allele frequencies, resulting in underestimation of N_e (Wang and Whitlock, 2003). Fortunately, recent methodological and theoretical developments allow N_es and migration rates to be estimated separately if reasonably large sample sizes and many markers are available (Wang and Whitlock, 2003). Nevertheless, if marine fish populations indeed have large N_es, changes in allele frequencies may be hard to estimate. Yet, the temporal approach still can provide useful insight about relative sizes of exploited and less exploited populations, as well as of the relative roles of migration and N_e in modulating divergence or lack thereof.

5. Conclusions

In conclusion, it is conceivable that fisheries management decisions based on the assessment of neutral genetic variability alone can lead to the loss of genetic biodiversity (i.e. locally adapted populations/stocks) due to the fact that neutral markers can fail to recognize locally adapted populations/stocks. Hence, sustainable fisheries management should invest more effort in evaluating the genetic basis for quantitative trait differentiation (e.g. age and size at maturity), and not rely solely on inference drawn from neutral genetic markers. This is in line with the *precautionary principle*, according to which the lack of scientific information on potentially adverse effects on genetic diversity should not be used as a reason to prevent or postpone measures to avoid or minimize such negative effects (FAO, 1996; Convention on Biological Diversity, 1992; Cartagena protocol on biosafety, 2000; Powles et al., 2000). In other words, clear phenotypic differences among fish - and perhaps even among marine mollusc - stocks should be considered as indicative of genetic differentiation until otherwise proven. This is also consistent with the principles of effective fisheries management in which distinct stocks are managed separately to optimize their yield.

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The resource status of Kumamoto oyster (*Crassostrea sikamea*)

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Natural Kumamoto oyster (*Crassostrea sikamea*) is thought to inhabit exclusively the Ariake Sea, Kyushu Island, Japan. This species has been of little importance in the Japanese fishery/aquaculture trade, yet it has been valued in the U.S. seafood market since the first exportation to U.S some sixty years ago. Owing to the inconspicuous morphological difference between Kumamoto oyster and the sympatric Pacific oyster (*C. gigas*) together with the phenotypic plasticity of oyster species in general, there was much taxonomic confusion between the two species. It is difficult, or rather, impossible based on the morphology alone to correctly assign oysters to the species thereby evaluating the resource status of Kumamoto oyster in its natural habitat has been hampered.

The advent of diagnostic molecular markers shed light on the taxonomic resolution between Kumamoto and Pacific oysters. Earlier molecular works relied on allozymes, as a large genetic divergence was uncovered between Kumamoto and Pacific oysters (Buroker et al, 1979). The progress of DNA sequencing and PCR (polymerase chain reaction) technologies has provided more efficient ways in which the species can unambiguously be determined: some diagnostic mitochondrial DNA markers have been developed, for example, PCR-RFLP (restriction fragment length polymorphism) for the large subunit ribosomal RNA mitochondrial gene (16S rRNA gene: Banks and Hedgecock, 1993; Banks *et al*, 1994) and single-tube multiplex PCR for the mitochondrial cytochrome oxidase I (COI) (Cordes *et al*, 2005; Wang and Guo, 2008). The property of maternal inheritance of mitochondrial molecules does not allow detecting possible occurrence of hybridization between Kumamoto and Pacific oysters, however, this problem can be resolved by the use of nucleotide DNA markers such as PCR-RFLP for the first internal transcribed spacer (ITS-1) of nuclear ribosomal RNA gene family (Hedgecock *et al*, 1999; Cordes *et al*, under review).

One public perception regarding the resource status of Kumamoto oyster is prevailing, that is to say, “Kumamoto oyster has become extinct or is threatened by extinction”. This is not correct. Repeated failures to find natural Kumamoto oyster, even equipped with some molecular markers, indeed suggested a possibility that the natural resource of Kumamoto oyster might be at stake (Banks et al, 1994; C. Langdon and J.P. Davis, pers. comm.). However, Hedgecock et al (1999) reported that 53 of *ca* 250 oysters derived from the Ariake, Yatsushiro, and Amakusa Seas yielded a concordant banding pattern of PCR-RFLP for the mitochondrial 16S rDNA and nuclear ITS-1 consistent with Kumamoto oyster, and Kumamoto oyster, rather than Pacific oyster, dominates certain sea shores. Usuki (2002) surveyed oysters collected from the northern Ariake Sea and confirmed the occurrence of Kumamoto oyster. Although the exact sample size at each location is not clearly shown, at some sites nearly 100% of individuals had mtDNA haplotype (PCR-RFLP of 16S rDNA, Banks *et al*, 1993) consistent with Kumamoto oyster. In contrary to Hedgecock *et al* (1999), he raised the possibility of natural hybridization between Kumamoto and Pacific oysters, which was however thrown out by Camara et al (2008). The abundance of Kumamoto oyster in the northern Ariake Sea without occurrence of Kumamoto/Pacific hybrid was confirmed by Camara et al (2008). The majority of their oyster samples (569 of 628 oysters) found to be Kumamoto oyster on the basis of multiplex PCR for mtDNA COI (Wang and Guo, 2008) coupled with PCR-RFLP for the nuclear ITS-1 (Cordes *et al*, under review), followed by the Suminoe oyster *C. ariakensis* (53). Pacific oyster only accounted for less than 1% of the samples (6 of 628). Given their results and rebuttals to Usuki (2002), they concluded that the species-lineage of bona fide Kumamoto oyster is reserved well in the northern Ariake Sea, and a massive amount of the resource are remained in the northern Ariake Sea: several millions of Kumamoto oyster is expected to reside just within one sampling site (Camara *et al*, 2008). In addition, the observation that some Pacific oyster microsatellite DNA (STRs: simple tandem repeats, nuclear DNA) markers subjected to cross-species amplification in natural Kumamoto oysters yielded no detectable allele, or alleles not shared between Kumamoto and Pacific oysters, also sets aside the possibility of Kumamoto/Pacific hybridization (M. Sekino, unpubl. data).

The argument on the taxonomy of Kumamoto oyster comes to an end with several lines of solid evidence from the molecular analyses. Kumamoto oyster should deserve a species status. Furthermore, Kumamoto oyster is neither rare nor threatened by hybridization with Pacific oyster, though the spatial and temporal coverage of field surveys needs to be extended to comprehend the habitat distribution. Such efforts will help establish an appropriate management strategy in conserving the biodiversity in the Ariake Sea.

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Relationship between the content of expansible 2:1 type clay minerals in paddy soils and the amount of ammonium nitrogen in the ponding water by a laboratory experiment

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Abstract

From the viewpoint of ammonium-nitrogen (N) transportation from paddy fields to rivers, estimation of ammonium-N in the ponding water is important for soil management to reduce N outflow from paddy fields. The relationship between the content of expansible 2:1 type clay minerals (i.e. smectite and vermiculite) and the amount of ammonium-N in the ponding water was investigated by a laboratory incubation experiment. Eighteen soils including fifteen alluvial soils and three volcanic ash soils from northeastern Japan were used; the clay content in soils was 116-476 g kg^{-1}; the content of expansible 2:1 type clay minerals in soils was 0-395 g kg^{-1}. The experimental condition corresponded to the condition of paddy fields just before transplanting, where readily available N fertilizer was incorporated to plow layers (0.15 m) at a rate 5 g N m^{-2} as basal fertilizer and submerged water depth was 0.06 m. The concentration and the amount of ammonium-N in the ponding water was 1.4-7.5 mg L^{-1} and 0.08-0.45 g m^{-2}. The amount of ammonium-N significantly related to the amount of clay in the calculated plow layer with a depth of 0.15 m (R^2=0.42). On the other hand, the amount of expansible 2:1 type clay minerals in the calculated plow layer more strongly related to ammonium-N than the amount of clay (R^2=0.55). These results were attributed to the fact that expansible 2:1 type clay minerals have larger amount of negative charge site per clay and higher selectivity in ammonium adsorption than other clay minerals. From these results it was considered that paddy soils rich in clay fraction or expansible 2:1 type clay minerals could adsorb larger ammonium and mitigate ammonium-N runoff through the ponding water.

1. Introduction

From the viewpoint of environment concern, nitrogen (N) outflow through the ponding water drainage from paddy fields is a serious problem, especially after application of readily available nitrogen fertilizer (Takamura et al., 1976; Asano et al., 1976). In a paddy field soon after basal fertilization and puddling, substantial part of total N in the ponding water was particle N (i.e. organic N and adsorbed ammonium-N in soil colloids), and dissolved ammonium-N occupied only about 20 % of total N, because large amount of suspended solids remain in the ponding water (Sato & Taguchi, 2000). However, the ponding water is usually drained several days after puddling for transplanting using machine. Dissolved ammonium-N was about 50 % of total N in the ponding water five days after puddling, when sedimentation of most suspended solids had been finished (Sato & Taguchi, 2000). Therefore, estimation of ammonium-N in the ponding water is important for soil management to reduce N outflow from paddy fields. It is reported that the percentage of adsorbed ammonium in soils ranges from 85 to 95 % in paddy fields (Okajima & Imai, 1973; Toriyama & Ishida, 1987). The residual ammonium-N is dissolved in the soil solution and ponding water. Clay minerals were main materials that adsorb ammonium ion in soils. Especially, expansible 2:1 type clay minerals (i.e. smectite and vermiculite) have high cation exchange capacity that is due to substantial isomorphic substitution and to the presence of fully expanded interlayers that promote exchange of ions, compared to the other clay minerals (Sparks, 1995). Smectite (Reid-soukup & Ulery, 2002) and vermiculite (Malla, 2002) have high adsorption selectivity of ammonium, compared to the other clay minerals. Ammonium adsorption selectivity is high in smectitic and low in allophanic soils (Okamura & Wada, 1984; Egashira et al., 1998). Therefore, it is considered that the content of smectite and vermiculite in soils strongly affects ammonium-N in the ponding water. However, no report that investigated the relationship between them is found. In the study, the relationship between the content of expansible 2:1 type clay minerals in paddy soils and the amount of ammonium-N in the ponding water was investigated by a laboratory incubation experiment.

2. Materials and Method

Eighteen soils were collected from northeastern Japan (thirteen soils from Miyagi Prefecture; two soils from Yamagata Prefecture; a soil from Akita, Niigata and Tochigi Prefecture, respectively). The soils used in this study were fifteen alluvial soils and three volcanic ash soils (Table 1). Clay content was determined by a pipette method (Nakai, 1997). Clay mineralogical composition of clay fraction was identified by X-ray diffraction (Rigaku Co. Ltd, Miniflex). Relative contents of the clay minerals were evaluated by using X-ray diffraction peak areas, according to the method of Egashira et al. (1995). Total contents of

Table 1 Properties of tested soils.

		In dry soil (g kg^{-1})							In calculated plow layer (kg m^{-2})			
		Clay	Smectite & Vermiculite in clay1)	Smectite & Vermiculite in soil	Total C	Total N	Acid oxalate extractable		Dry soil2)	Clay	Smectite & Vemiculite	Total C
							Fe	Al				
Alluvial soils	Tsuruoka	116	357	41	25.7	2.4	2.2	3.5	113	13.2	4.7	2.9
	Aotsuka	262	672	176	31.2	2.7	8.8	1.5	95	24.8	16.7	3.0
	Ishikoshi I	262	716	187	20.8	1.9	12.9	2.5	90	23.5	16.8	1.9
	Fujishima	264	595	157	37.8	3.3	10.8	2.8	79	20.7	12.4	3.0
	Tajiri	292	651	190	20.3	1.8	11.8	1.6	82	23.8	15.5	1.7
	Ishikoshi II	315	743	234	22.7	2.1	12.5	1.6	89	28.1	20.9	2.0
	Nakada	327	749	245	15.4	1.4	14.2	1.7	90	29.5	22.1	1.4
	Furukawa	359	928	333	36.5	3.2	15.7	1.7	81	29.0	26.9	2.9
	Hasama	385	764	294	23.6	2.2	13.2	1.6	78	29.8	22.8	1.8
	Iijima	388	697	270	30.7	2.9	11.1	1.7	73	28.5	19.9	2.3
	Kiyosato	411	961	395	39.3	4.0	16.7	3.3	74	30.5	29.3	2.9
	Ohgata	476	679	323	41.6	4.3	7.3	2.5	61	29.0	19.7	2.5
	Minamikata muck	345	465	161	70.6	4.9	14.3	3.4	74	25.4	11.8	5.2
	Toyosato muck	347	373	129	51.4	4.3	8.5	2.9	76	26.4	9.9	3.9
	Nakada muck	373	416	155	73.0	5.4	12.1	2.7	69	25.9	10.8	5.1
Volcanic ash soils	Naruko	126	401	51	46.7	3.7	5.4	9.1	103	13.0	5.2	4.8
	Sikama	211	530	112	68.8	5.2	10.0	20.0	79	16.8	8.9	5.5
	Mohka	341	0	0	106.6	8.1	14.2	29.8	76	25.7	0.0	8.0

1) Evaluated using X-ray diffraction peak areas, according to Egashira et al. (1995).

2) Dry weights in plow layers with depth of 0.15 m.

carbon (C) and N were determined by a dry combustion method (Sumitomo chemical Co. Ltd., NC-80S). Acid oxalate (pH 3.0) extractable iron and aluminum were determined by atomic adsorption spectrometry, according to the method of Ito (1997).

Moist soils without drying after collection from paddy fields were taken into glass bottles (volume, 100 mL; base area, 0.0010 m^{-2}) to adjust the sedimentation height to 50 mm (±2 mm). The weight of used soils per each bottle ranged from 20.3 to 37.9 g (dry soil). Ammonium chloride was added at a rate 1.67 g N m^{-2}. Deionized water was added to dip the soil clods, and the bottles were shaken for 30 minutes. Water level was adjusted to 70 mm from the bottom, and the bottles were incubated at 20 °C for 5 days. This experimental condition corresponded to the condition of paddy fields just before transplanting, where readily available N fertilizer was incorporated to plow layer (0.15 m) at a rate 5 g N m^{-2} as basal fertilizer under submerged water depth of 0.06 m. After the incubation, the ponding water with a depth of 0.02 m in the bottle was taken by using a pipette and filtered with a membrane filter (0.45 μm). The concentration of ammonium in the ponding water was determined by the indophenol blue photometric method (Scheiner, 1976).

3. Results and Discussion

Table 1 shows some chemical and clay mineralogical properties of tested soils. The clay content in soils was 116-476 g kg^{-1}. The content of expansible 2:1 type clay minerals was 0-395 g kg^{-1}. The contents of total clay and expansible 2:1 type clay minerals correspond to 13-30 kg m^{-2} and 0-29 kg m^{-2}, respectively, when the laboratory experimental condition is extended to the field condition with plow layer depth of 0.15 m. Acid oxalate extractable aluminum was large in three volcanic ash soils, and Mohka soil is dominant in allophanic clay.

Fig. 1 shows the relationship between the amount of clay, and expansible 2:1 type clay minerals in the calculated plow layer with a depth of 0.15 m (the calculated plow layer, hereafter) and the amount of ammonium-N in the ponding water. The concentration and amount of ammonium-N in the ponding water were 1.4-7.5 mg L^{-1} and 0.08-0.45 g m^{-2}. 3-9 % of added ammonium-N (5 g N m^{-2}) was dissolved in the ponding water. There was a significant negative relationship between the amount of clay in the calculated plow layer and the amount of ammonium-N in the ponding water (R^2=0.42). The result indicated that soils with larger amount of clay had higher negative charge and adsorbed larger amount of ammonium ion. However, the amount of ammonium-N in the ponding water showed much variation in soils with the similar amount of clay. The variation of the amount of ammonium-N in the ponding water is considered to be due to ammonium adsorption capacity with the different clay minerals and ammonium adsorption by humus in the soils.

As shown in Fig. 1 (right side), the amount of expansible 2:1 type clay minerals in the calculated plow layer showed closer correlation with the amount of ammonium-N in the ponding water than those of

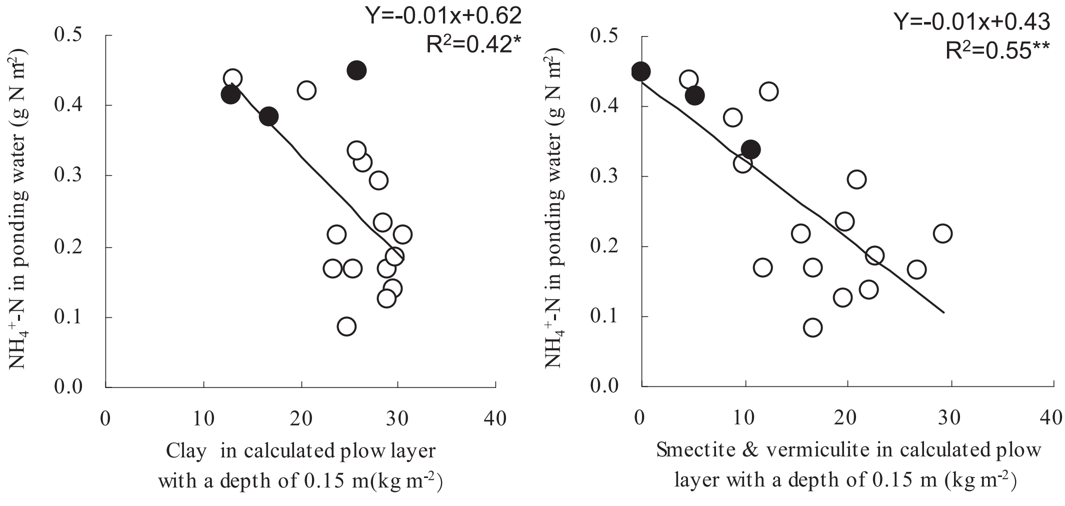

Fig. 1 Relationship between clay, expansible 2:1 type clay minerals (smectite and vermiclute) in the calculated plow layer and NH_4^+-N in surface water. Asterisks mean the relationship was significant (n=18; * p<0.01; ** P<0.001). ○ : alluvial soil, ● : volcanic ash soil.

clay (R^2=0.55). These results were attributed to the fact that expansible 2:1 type clay minerals had larger amount of negative charge site per clay (Sparks, 1995) and higher selectivity in ammonium adsorption than the other clay minerals (Reid-soukup & Ulery, 2002; Malla, 2002).

Soil organic matter (SOM) also possesses ammonium adsorption ability because of its negative charge. However, coefficient of determination (R^2) between the amount of C in the calculated plow layer and ammonium-N in the ponding water was no more than 0.22, although the relationship was significant ($p<0.05$). It is considered that a substantial part of the relationship was indirect, because there was a significant relationship between the amount of C and expansible 2:1 type clay minerals in the calculated plow layer (R^2=0.50). Therefore, the effect of ammonium adsorption of SOM on the amount of ammonium-N in the ponding water was not clarified in the study.

From these results obtained by the laboratory experiment conducted under the condition similar to paddy fields, it was considered that paddy soils rich in clay fraction or expansible 2:1 type clay minerals could adsorb larger ammonium and mitigate ammonium-N runoff through the ponding water drainage from paddy fields.

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Effects of available nitrogen and ammonium adsorption of plow layer on nitrogen uptake and yield of paddy rice (*Oryza sativa* L.)

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Abstract

Effects of available nitrogen (N) and ammonium adsorption of plow layer (PL) on N uptake and brown rice yield were investigated for seven soils. The experiment was conducted in a single paddy field under same conditions except for soils. Rice plants were grown in frames, which the seven different soils (four alluvial paddy soils and three andic paddy soils) were repacked. Not only a small percolation condition (i.e. original subsoil (OS) plot) but also an increased percolation condition was treated by the replacement of subsoil with sand (i.e. sandy subsoil (SS) plot). Percolation rate was lower in clayey smectitic (1-2 mm day^{-1}) than in sandy and andic (5-9 mm day^{-1}) soils in the SS plots. In the OS plots, percentage of exchangeable ammonium-N in total soil ammonium N was 89-96 % in all soils at 11 days after transplanting (DAT), and was higher in soils with the large ammonium adsorption of PL. Soil ammonium-N at 40 DAT (i.e. at active-tillering stage), N uptake at harvest and brown rice yield were explained by the available N of PL in the OS plots. It is considered that the difference of ammonium adsorption of PL did not strongly affect them, because of its small variation and the small percolation. In the SS plots, the soil ammonium-N at 40 DAT was explained by the ammonium adsorption and the available N of PL. It is considered that soil ammonium-N was much protected from leaching and denitrification in soils with the large ammonium adsorption of PL. However, the total N uptake and the brown rice yield were effectively explained by the available N of PL, and the ammonium adsorption of PL was not selected as an important parameter. It is considered that the ammonium adsorption of PL did not strongly affect the N uptake after maximum tillering stage. The ammonium adsorption of PL did not significantly related to recovery rate of fertilizer N both in the OS and SS plots because of the small variation in the ammonium adsorption of PL among soils.

INTRODUCTION

Yield of rice (*Oryza sativa* L.) is related to the amount of nitrogen (N) uptake until maturity (Wada, 1969). In the Tohoku district of Japan, 70 % of the total N uptake by rice plants comes from mineralized soil organic N (Shoji & Mae, 1984), especially from that in plow layers (PL) (Ando et al., 1990). Therefore, the amount of mineralization of soil organic N (available N) in PL profoundly affect the N uptake by rice plant. On the other hand, 30 % of the total N uptake by rice plant comes from fertilizer N. Recovery rate of fertilizer N with basal application was in the range from 18 % to 42 %. About 30 % of basal fertilizer N is immobilized in soils and about 40 % is diminished by denitrification and leaching (Shoji & Mae, 1984). Average of the recovery rate of topdressed fertilizer N was about 55 % (Shoji & Mae, 1984). From these studies, the recovery rate of the sum of basal and topdressed fertilizer N (5 g N m^{-2} and 2 g N m^{-2}, respectively) is estimated at between 30 and 46 %.

It is generally considered that ammonium adsorption in soil can affect the N uptake by rice plant. It is reported that the percentage of adsorbed ammonium in soils ranges from 85 to 95 % in paddy fields (Okajima & Imai, 1973; Toriyama & Ishida, 1987). The selectivity of ammonium is high in smectitic and low in allophanic soils (Okamura & Wada, 1984; Egashira et al., 1998). In a column experiment, leaching rate of ammonium was extremely less in smectitic soils than those in soils rich in allophane (Harada & Kutsuna, 1960). In a pot experiment, tillering of rice plants at early tillering stage was less in a smectitic soil than those in soils rich in other crystalline clay minerals. However, at the end of the tillering stage, the tiller number increased more rapidly and the yield of grain was higher in smectitic, than in the other soils (Harada et al., 1960). The recovery rate of basal fertilizer nitrogen was higher in a soil rich in smectite than in a soil rich in 2:1-2:1:1 intergraded minerals (Shoji et al., 1976). It is generally considered that N deficiency often occurs in coarse textured alluvial and andic paddy soils because of large amount of N leaching (Wakatsuki, 1998). These studies suggest that the ammonium adsorption by soil will influence the fertilizer N recovery and rice growth.

However, it is still questionable if the difference in the ammonium adsorption of soils affects the N uptake and the rice yield in the fields. The amount of fertilizer N applied in the column leaching experiment by Harada and Kutsuna (1960) was extremely greater than under condition of actual rice cultivation in fields. In a lysimeter experiment, which fertilizer N was applied at almost the same rate as for actual rice cultivation, the amount of N leaching was only 2 % of the applied fertilizer N (Maeda & Onikura, 1976). No relationship between the cation exchange capacity and the amount of N uptake by rice plants was observed among five paddy soils in Yamagata Prefecture (Tanaka et al., 1982).

The effects of the available N and the ammonium adsorption of PL on the N uptake and the grain yield of rice plants have not been comprehensively investigated in a field. In this study, we investigated if the ammonium adsorption of PL affects the N uptake and the yield of rice, together with the available N. In connection with ammonium-N in soils, an increased percolation promotes the ammonium-N leaching. It is also considered that oxygen supplied with percolating water promotes nitrification and denitrification. Therefore, it is hypothesized that the positive effects of the ammonium adsorption on the N uptake and the rice yield are clearly observed under the increased percolation condition. The effects of the ammonium adsorption were investigated also under a condition, where the percolation was increased and the rice plants depended N uptake only on the PL.

MATERIALS AND METHODS

1. Soils

PL of four alluvial and three andic paddy soils from the Tohoku district of Japan were used (Table 1). Fujishima soil was used only in 2005, and the other soils were used in 2004 and 2005 without resampling. Clay content was determined by a pipette method. Clay mineralogical composition of clay fraction was identified by the X-ray diffraction (Rigaku Co. Ltd, Miniflex). Total contents of carbon and N were determined by a dry combustion method (Sumitomo chemical Co. Ltd., NC-80). The available N was evaluated by incubation using the fresh moist soils under a submerged condition at 30 ˚C for 4 weeks. The ammonium adsorption was estimated in a laboratory experiment. 10 g of the air-dry soils (<2 mm) was added with 20 mL of 25 mg N L^{-1} ammonium chloride (containing nitrapyrin at a concentration of 2 mg L^{-1} as nitrification suppressor) and was shaken for 24 h at 25 ˚C. The amount of added ammonium-N corresponded to 5 g N m^{-2} in the field. Ammonium chloride was used to avoid the effects of pH change or salt adsorption, which was expected to occur by the addition of ammonium carbonate or ammonium sulfate. After shaking, the concentration of ammonium-N in the filtered solution was determined by the indophenol blue photometric method (Scheiner, 1976). The decrease of ammonium-N in the solution was regarded as the ammonium adsorption rate by the soil. Therefore, the ammonium adsorption rate evaluates the sum of exchangeable form and fixed form into 2:1 type clay minerals. The available N and the ammonium adsorption of PL per square meter were calculated, using the weight of dry soil in the PL (0.15 m).

Table 1 Properties of the tested soils.

Soils		Clay content	Dominated clay minerals†	T-C	T-N	Available N‡		NH_4^+-N adsorption rate	Weight of dry soil in the plow layer (PL§)	Available N in PL		NH_4^+-N adsorption of PL‡
						2004	2005			2004	2005	
		(g kg^{-1})		(g kg^{-1})	(g kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(kg m^{-2})	(g m^{-2})	(g m^{-2})	(g m^{-2})
Alluvial	Tsuruoka	116	Sm, Int, KM	25.7	2.4	31.8	18.9	32.6	165	5.3	3.1	5.4
soils	Fujishima	264	Sm, Int, KM	37.8	3.3	-	60.5	41.0	126	-	7.6	5.2
	Kiyosato	411	Sm	39.3	4.0	65.4	33.0	44.7	125	8.2	4.1	5.6
	Ohgata	476	Sm, Int	41.6	4.3	60.2	46.7	47.2	118	7.1	5.5	5.6
Volcanic	Naruko	126	Sm, Vt, Int, Ch, KM	46.7	3.7	30.6	18.3	36.1	120	3.7	2.2	4.3
ash soils	Shikama	211	Sm, Int	68.8	5.2	26.6	21.8	38.0	113	3.0	2.5	4.3
	Mohka	341	Amor	106.6	8.1	20.8	17.2	38.4	106	2.2	1.8	4.1

† Abbreviations; Sm, smectite; Int, 2:1~2:1:1 intergraded minerals; KM, kaolin minerals; Vt, vermiculite; Ch, chlorite; Amor, amorphous minerals.

‡ Available N was evaluated by incubation under anaerobic condition at 30 °C for 4 weeks, using fresh moist soils before fertilization and puddling in each year.

§ The values were calculated using the weights of dry soil in the plow layers (0.15 m depth).

2. Replacement of PL by the soils and cultivation method

The field experiment was conducted at a paddy field in Field Science Center, Graduate School of Agricultural Science, Tohoku University, Miyagi Prefecture, the Tohoku district of Japan (38°44’ N; 140°15’ E). The paddy soil was Wet Andosol (Typic Melanudands) on terraced land. The depth of the PL was 0.15 m. The percolation rate was 3 mm day^{-1} at the most, because of the artificial subsoil compaction. In 2004 and 2005, plastic bottomless frames (0.076 m^2, 0.28 m×0.28 m×0.18 m height) were buried in the PL and soil in the frames was removed (i.e. original subsoil (OS) plot). In 2005, the effects of the ammonium adsorption of PL were investigated also in a condition, where the percolation was increased and rice did not elongate the roots to the subsoil. The subsoil (0.15-0.70 m in depth) was replaced with sand in a part of the field (7 m×1 m). The half-size frames (0.038 m^2, 0.28 m×0.14 m×0.18 m height), with fabric for root zone restriction attached on the bottom were buried in the plow layer (i.e. sandy subsoil (SS) plot). The fabric was made of polyester, and water can percolate through it (percolation rate was 10^{-4} cm s^{-1} in an upland condition, measured by the company). To evaluate the N uptake from the subsoil, additional circle frames (0.048 m^{-2}) with and without the fabric on the bottom were buried on the original subsoil. On May 12, 2004 and May 14, 2005, each PL was puddled with chemical fertilizers and rice straw. Each soil was filled in the frames to a depth of 0.15 m. Urea was incorporated into the PL at a rate of 5 g N m^{-2} as basal dressing and was topdressed at a rate of 2 g N m^{-2} at panicle initiation stage using ^{15}N labeled urea (3.05 atom %). Potassium chloride (7 g K_2O m^{-2}), calcium phosphate (7 g P_2O_5 m^{-2}) and rice straw (500 g dry matter m^{-2}) were also incorporated into the PL as the basal application. The plow layer of the field, which was applied with mixed fertilizer (7 g N m^{-2}; 10 g K_2O m^{-2}; 7 g P_2O_5 m^{-2}), was filled into the additional circle frames. Rice seedlings (cultivar: Hitomebore) at 7 leaf age were transplanted on May 14, 2004 and on May 17, 2005. Each hill was comprised of three seedlings. Two hills or one hill was transplanted in a frame in the OS plots or the SS plots. The rice plants were also grown around the frames. Planting density was 22.2 hills per m^2 in the vegetation community around the frames and 26.4 hills per m^2 in the frames. In the additional circle frame, two hills were transplanted, and the planting density was 41.7 hills per m^2. Midseason drainage was performed from July 8 to 12 in 2004 and from July 9 to 12 in 2005. Topdressing at the panicle initiation stage was applied on July 13, 2004 and on July 14, 2005. The rice plants above ground were harvested on September 18, 2004 and September 21, 2005.

3. Methods of field survey and chemical analysis

The percolation rate was measured in 2006, a year after the rice cultivation experiment. Plastic cylinders (diameter 0.07 m, height 0.33 m) with a small pore (diameter 0.01 m) were inserted to a depth of 0.20 m, and the PL inside the cylinders was removed. The seven soils were puddled with the chemical fertilizers at the rate of the basal fertilization in the cultivation experiment. The soils were packed in the cylinders to a depth of 0.15 m in the SS plot. The cylinders packed with Naruko soil were settled also in the OS plot. The small pores on the side were usually open so that ponding water level was same inside and outside the cylinders. The pores were closed with stoppers periodically, and the percolation rate was measured with a decrease of ponding water level after a day. The experiment was carried out with three replications.

The ammonium-N in the PL was measured at 3, 14, 35 and 48 days after transplanting (DAT) in 2004 and at 11 and 40 DAT in 2005. Soil samples were taken at two or four points in a frame to a depth of 0.12 m using a small cylinder (35 mL volume). The soil samples collected from the same frame were mixed and the soil samples of 30 g were centrifuged at 10,000 rpm for 20 minutes and the supernatant was obtained as soil solution. Exchangeable ammonium-N was extracted by shaking the precipitated soils for 2 hours with 2 M potassium chloride (KCl). The concentration of ammonium-N in the soil solution and the KCl extractant was determined by the indophenol blue photometric method (Scheiner, 1976). The amounts of ammonium-N per unit area (g N m^{-2}) in the soil solution (i.e. dissolved) and in the exchangeable form were calculated using the concentrations of ammonium-N and weight of dry soil in each frame.

The tiller number was measured periodically. After harvest, the shoots and half of the grains were dried in an oven at 70 ˚C for more than 72 hours and their dry weights were determined. The N content was determined by a dry combustion method (Sumitomo chemical Co. Ltd., NC-80). The ^{15}N atom percentages in the shoots and the grains were determined with a mass spectrometer (Finnigan MAT, DELTA plus). The N uptake from the fertilizer was determined using the ^{15}N ratio and the N concentration. The brown rice yield and the yield components were determined using the other half of the grains. The ripened grains and the poorly ripened grains were separated with sodium chloride solution with a density of 1.06. The thousand-grain weight and the brown rice yield were calculated to have a grain water content of 15 %.

4. Statistical Analysis

N supply from soil was largely different between the OS and SS plots, because of the percolation and the lack of root elongation to the subsoil. Therefore, statistical analysis was conducted separately in each plot. The experiment was carried out with triplicate randomized plots. Analysis of variance among soils was carried out using Tukey-Kramer's method. The effects of the available N and the ammonium adsorption of PL on the ammonium-N in PL at the tillering stage, the grain number, the brown rice yield and the N uptake in 2005 were investigated by multiple regression analysis (step-wise selection). In the step-wise selection, the available N and the ammonium adsorption of PL were selected as the parameters using p-value of 0.25. JMP 4.0.5. J (SAS Institute Inc.) was used for all analysis of variance and multiple regression analysis.

RESULTS AND DISCUSSION

1. Soil properties

Properties of the tested soils are shown in Table 1. The ammonium adsorption rate per the soil weight was larger in clayey smectitic soils (Ohgata and Kiyosato soils) than sandy soil (Tsuruoka soil). However, the difference of the ammonium adsorption of PL was not large among the alluvial soils, because the weight of dry soil in the PL was larger the in the sandy soil. The available N of PL was large in the alluvial soils. The available N of PL was larger in 2004 than in 2005; this is attributed to the positive effect of crushing and moderate drying during pretreatments in 2004.

2. Percolation rate in each soil

Table 2 shows the average percolation rate in each soil in 2006, a year after the cultivation experiment. The average percolation rate was only 1 mm day^{-1} in Naruko soil in the OS plots. On the other hand, it was 9 mm day^{-1} in Naruko soil in the SS plots. This difference was because of the subsoil modification. It is considered that the percolation rate was large also in 2005, because the difference between the OS and SS plots was clear even a year after the subsoil modifica-

Table 2 The average percolation rate in each soil during the irrigation season.

Subsoil replacement and plow layers.	pecoration rate (mm day^{-1})
Original subsoil (without subsoil replacement)	
Naruko	0.4 ± 0.0
Sandy subsoil (with subsoil replacement).	
Tsuruoka	5.2 ± 0.3
Fujishima	2.1 ± 0.2
Kiyosato	1.3 ± 0.2
Ohgata	2.3 ± 0.3
Naruko	8.7 ± 1.4
Shikama	7.8 ± 0.3
Mohka	8.6 ± 0.1
sand[‡]	39.9 ± 1.8

† Variation shows standard error (n=3).
‡ The sand is similar to that was used for subsoil replacement.

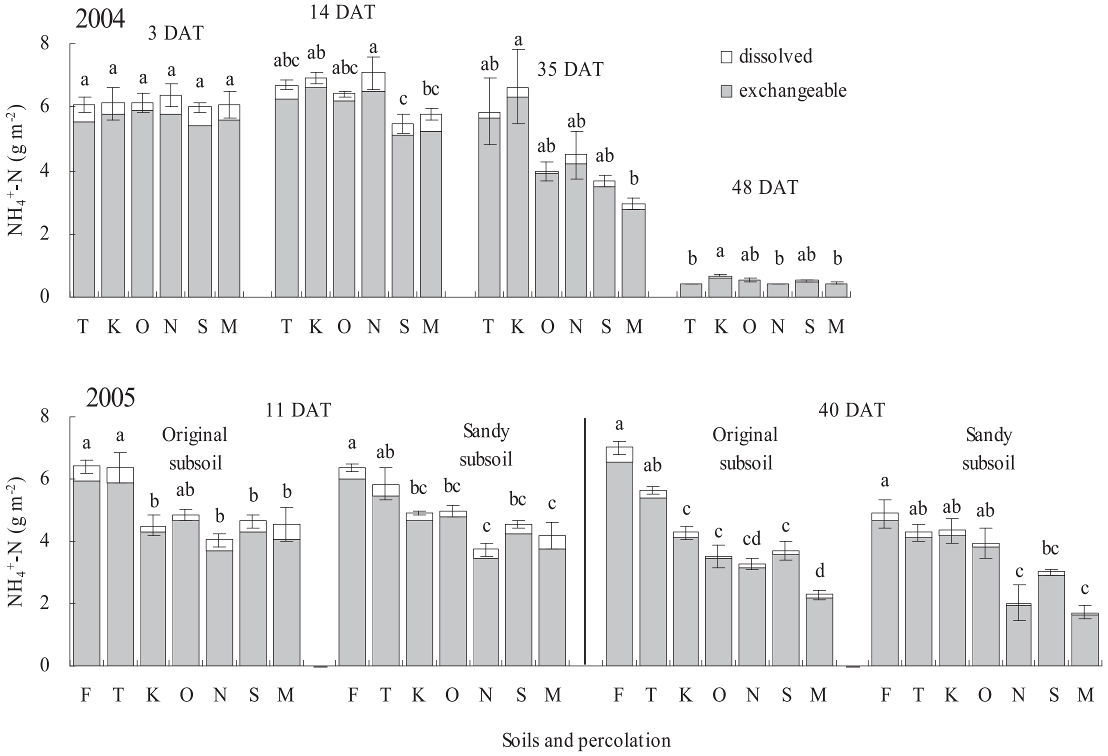

Fig. 1 The amounts of dissolved and exchangeable NH_4^+-N in the plow layers. Abbreviations: F, Fujishima; T, Tsuruoka; K, Kiyosato; O, Ohgata; N, Naruko; S, Shikama; M, Mohka. Error bars show standard error of total NH_4^+-N (n=3). Different letters show that means of total soil NH_4^+-N differ significantly ($p<0.05$, Tukey-Kramer's method).

tion. In the SS plots, the percolation rate was larger in the three andic soils than in the four alluvial soils. Among the alluvial soils, the percolation rate was small in Fujishima, Kiyosato and Ohgata soils (1-2 mm day^{-1}), which possessed large clay content. The percolation rate of sandy subsoil was 40 mm day^{-1}. Therefore, it is considered that the percolation rate (1-9 mm day^{-1}) approximately corresponded to those in ordinary paddy soils without artificial subsoil compaction in the Tohoku district of Japan.

3. Amount of ammonium-N in the PL.

The amounts of dissolved and exchangeable ammonium-N in the PL are shown in Figure 1. Total soil ammonium-N is defined as the sum of dissolved and exchangeable ammonium-N. Shoji et al. (1971) reported that ammonium-N from basal fertilizer (6-7 g m^{-2}) was only 15-38 % of total soil ammonium-N at 24-25 DAT, largely because of mineralization of soil organic N. Therefore, it is considered that total soil ammonium-N was derived from not only fertilizer N but also soil N in this study.

In the OS plots at 3 DAT in 2004, the percentage of exchangeable ammonium-N in total soil ammonium-N (defined as percentage of exchangeable ammonium-N) was no less than 90-96 %. We suggested that soil colloids could adsorb almost all of the added ammonium-N just after application of fertilizer, because the soil ammonium-N was much less than the negative charge of the soil. However, the percentage of exchangeable ammonium-N was slightly higher at, respectively, 96 % and 94 % in Ohgata and Kiyosato soils, clayey and smectitic, than those in the other soils. At 35 DAT, the total soil ammonium-N was 3.0 g m^{-2} and significantly smaller in Mohka soil than in the other soils. Until 48 DAT, the total soil ammonium-N rapidly decreased to 0.4-0.7 g m^{-2} in all soils. The rapid decrease was largely attributed to the uptake of fertilizer and soil N by rice plants.

Also at 11 DAT in 2005 in the OS plots, the percentage of exchangeable ammonium-N ranged from 90 % to 96 % in all soils. Figure 2 shows the significant linear relationship between the ammonium adsorption of PL estimated by a laboratory experiment and the percentage of exchangeable ammonium-N in the field. We suggested that the ammonium adsorption of PL determined by the incubation experiment was effective in estimating existing forms in the paddy fields after fertilizer application. At 40 DAT in the OS plots, the total soil ammonium-N was high in Fujishima soil with 7.0 g m^{-2} and was the smallest in Mohka soil with 2.3 g m^{-2}.

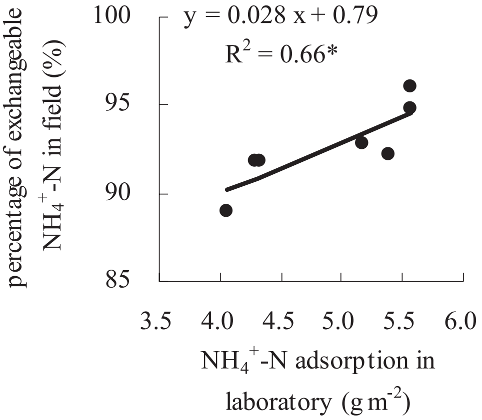

Fig.2 The relationship between the ammonium adsorption of plow layer (g m^{-2}) in a laboratory experiment and the percentage of exchangeable NH_4^+-N in total soil NH_4^+-N (%) in a field at 11 DAT in the original subsoil plot in 2005. * means the relationship is significant ($p<0.05$).

At 11 DAT, the dissolved ammonium-N in the SS plots was less than that in the OS plots, in Tsuruoka, Fujishima, Naruko, Shikama and Mohka soils. We considered that the percolation increased leaching and denitrification of ammonium-N. However, the differences were the least in Ohgata and Kiyosato soil rich in smectitic clay, probably because of the large ammonium adsorption of PL and the small percolation. At 40 DAT, the total soil ammonium-N was less in the SS plots than the OS plots in Tsuruoka, Fujishima, Naruko, Shikama and Mohka soils. On the other hand, there was almost no difference in the total soil ammonium-N in Ohgata and Kiyosato soils. It is considered that leaching and denitrification of ammonium-N were less in the clayey smectitic soils than in the other soils.

4. Tiller number change of rice

The tiller number change was shown in Figure 3. In the OS plots, the tiller number was the highest in Kiyosato soils in 2004 and Fujishima soils in 2005, and was the lowest in Mohka soil. Also in the SS plots in 2005, the tiller number was the highest in Fujishima soil and the lowest in Mohka soil, similarly to the OS plots.

5. N uptake and recovery rate of fertilizer N

Figure 4 shows the N uptake by the rice plants from soil and fertilizer, and the recovery rate of fertilizer N at the harvest. Both in the OS and SS plots, the N uptake from soil largely attributed to the total N uptake. The total N uptake was closely related to the N uptake from soil (R^2=0.99 in 2004; R^2=0.99 in 2005 in the OS plots; R^2=0.97 in the SS plots).

In the OS plots, the total N uptake was the largest in Kiyosato soil with 12.6 g m^{-2} in 2004 and Fujishima soil with 15.1 g m^{-2} in 2005, and was small in Mohka soil. The recovery rate of fertilizer N ranged from 28 % to 33 %, and the difference among every soil was

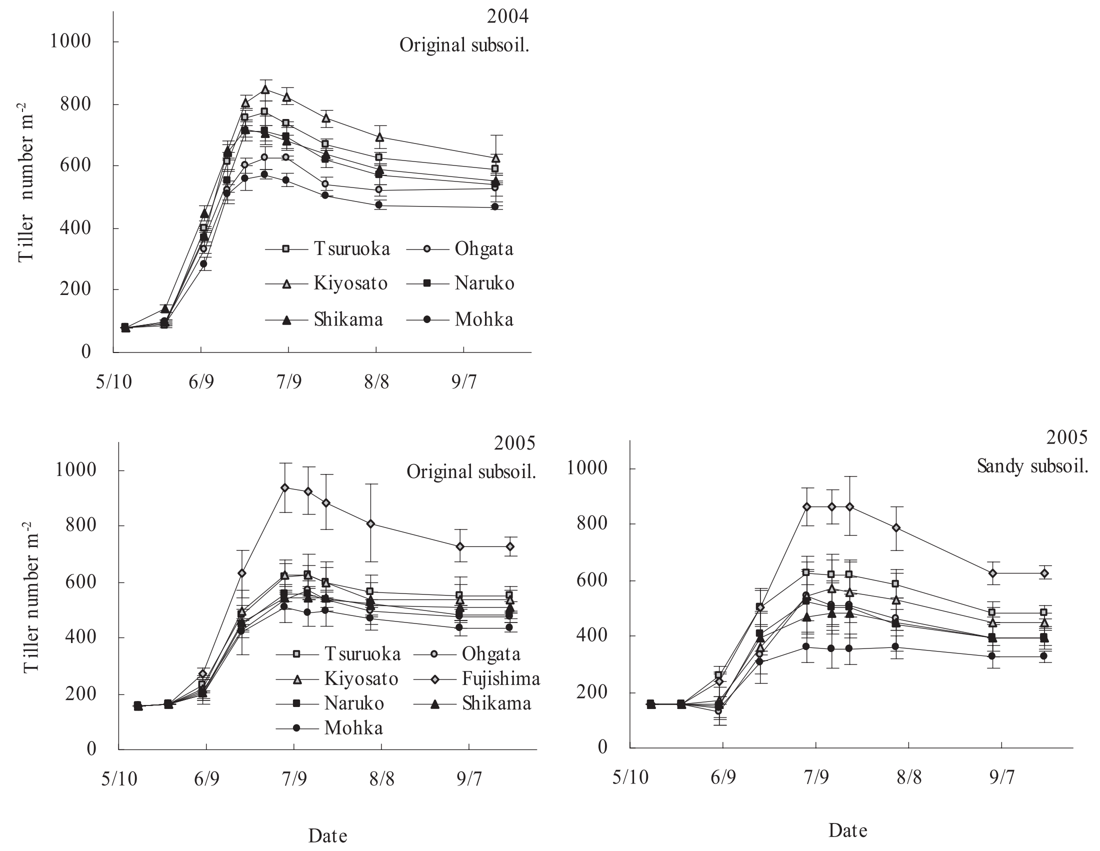

Fig. 3 The change of tiller number. Error bars show standard error (n=3).

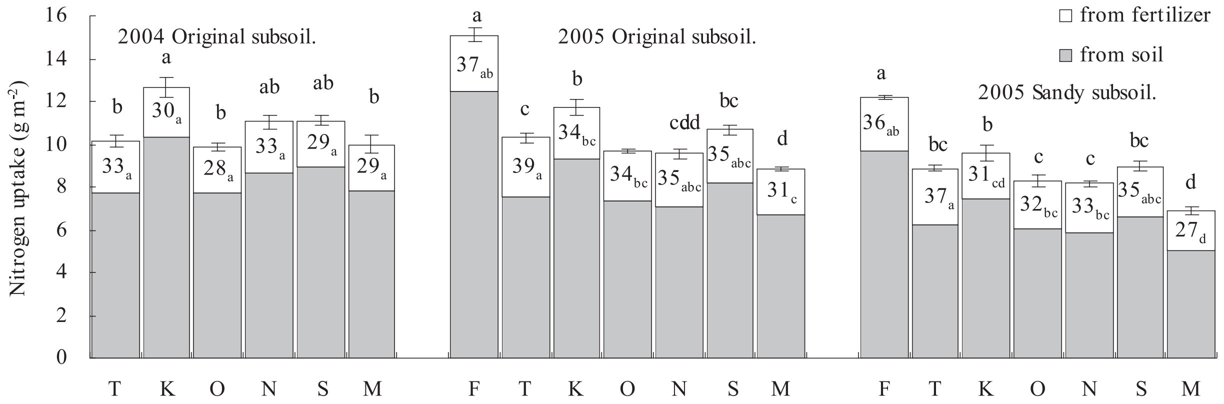

Fig. 4 The Nitrogen uptake and the recovery rate of fertilizer nitrogen at the harvest. Numbers in the figure shows the recovery rates of fertilizer nitrogen (%). Abbreviations: F, Fujishima; T, Tsuruoka; K, Kiyosato; O, Ohgata; N, Naruko; S, Shikama; M, Mohka. Error bars show standard errors of the total nitrogen uptake (n=3). Different letters show that total nitrogen uptakes and the recovery rates differ significantly between soils in each year and percolation plot ($p<0.05$, Tukey-Kramer's method).

not significant in 2004. The recovery rate of fertilizer N was the highest in Tsuruoka soil with 39 % and the lowest in Mohka soil with 31 % in 2005. The recovery rate approximated to 30-46 % calculated from previous studies (Shoji & Mae, 1984). The N uptake was smaller in the SS plots than in the OS plots. According to the additional cultivation experiment using the circle frames, the difference of the N uptake was 2 g m^{-2} between the plots with and without the fabric (data is not shown). Therefore, the smallness of N uptake in the SS plots was largely due to the lack of N uptake from subsoil. The total N uptake was the largest in Fujishima soil with 12.2 g m^{-2} and the smallest in Mohka soil with 6.9 g m^{-2} in 2005. The recovery rate was larger in Tsuruoka soil than in Kiyosato and Ohgata soil, and was not significantly related to the ammonium adsorption of PL. This was probably due to the small variation in the ammonium adsorption of PL among soils.

6. Brown rice yield and yield components

Table 3 shows the brown rice yield and the yield components. In the OS plots, the brown rice yield was significantly large in Kiyosato soil (799 g m^{-2}) in 2004 and in Fujishima soil (950 g m^{-2}) in 2005, and small in Mohka soil (614 g m^{-2} in 2004; 531 g m^{-2} in 2005). In the SS plots in 2005, the brown rice yield was also the highest in Fujishima soil (791 g m^{-2}) and the lowest in Mohka soil (396 g m^{-2}). The brown rice yield was significantly related to the total N uptake (R^2= 0.63 and R^2=0.93 in 2004 and 2005 in the OS plots; R^2=0.85 in the SS plots in 2005).

The brown rice yield was strongly related to the grain number (R^2=0.87 and R^2=0.99 in 2004 and 2005 in the OS plots; R^2=0.98 in 2005 in the SS plots), because variations in the percentage of ripened grains and the thousand-grain weight were not so great among all soils (88-95 % and 21.4-23.0 g m^{-2}). In the OS plots, the panicle number was significantly small in Mohka soil in 2004, and was significantly large in Fujishima soil in 2005. There was no significant difference in the grain number per ear between soils in 2004 and 2005. In the SS plots in 2005, the panicle number was significantly large in Fujishima soil and small in Mohka soil. As shown in Figure 5, the amount of total soil ammonium-N in the PL at 40 DAT in 2005 were significantly correlated with the grain number, which related most strongly to the brown rice yield. The total soil ammonium-N in PL at 35 or 40 DAT was also significantly related to the maximum tiller and the panicle number both in the OS and SS plots. Tanaka et al. (1982) found a rela-

Table 3 The brown rice yield and yield components.

Year and subsoil	soils	Brown rice yield (g m^{-2})	Panicle number (m^{-2})	Number of grains per ear	Number of grains (× 10^{3} m^{-2})	Percentage of ripened grains (%)	Thousand-grain weight (g)
2004 Original subsoil	Tsuruoka	649 ±36 b	591 ±36 a	56 ±4 a	33.1 ±1.3 b	88 ±4 a	22.4 ±0.2 ab
	Kiyosato	799 ±20 a	626 ±29 a	62 ±2 a	38.6 ±1.2 a	93 ±2 a	22.2 ±0.2 b
	Ohgata	691 ±13 b	529 ±23 a	64 ±2 a	33.6 ±0.3 b	91 ±2 a	22.6 ±0.1 ab
	Naruko	687 ±27 b	538 ±16 a	59 ±2 a	31.7 ±1.1 bc	94 ±2 a	22.9 ±0.0 a
	Shikama	660 ±1 b	555 ± 8 a	56 ±1 a	30.7 ±0.1 bc	93 ±2 a	22.9 ±0.1 ab
	Mohka	614 ±8 b	467 ± 4 b	61 ±1 a	28.2 ±0.3 c	94 ±1 a	23.0 ±0.1 a
2005 Original subsoil	Fujishima	950 ±10 a	727 ±33 a	64 ±3 a	46.4 ±0.3 a	94 ±0 a	21.7 ±0.0 a
	Tsuruoka	668 ±27 b	551 ±22 b	62 ±1 a	34.0 ±1.0 b	91 ±1 a	21.7 ±0.1 a
	Kiyosato	687 ±22 b	538 ±46 b	61 ±3 a	32.5 ±1.3 b	95 ±1 a	22.4 ±0.3 a
	Ohgata	652 ±15 b	476 ±23 b	68 ±3 a	32.1 ±0.6 b	92 ±0 a	22.1 ±0.2 a
	Naruko	580 ±17 c	480 ±12 b	60 ±1 a	28.7 ±0.3 cd	91 ±2 a	22.1 ±0.1 a
	Shikama	646 ±2 b	507 ±23 b	62 ±3 a	31.5 ±0.2 bc	93 ±1 a	22.0 ±0.2 a
	Mohka	531 ±7 d	436 ±15 b	59 ±1 a	25.9 ±0.3 d	94 ±1 a	21.9 ±0.1 a
2005 Sandy subsoil	Fujishima	791 ±17 a	626 ±23 a	63 ±1 a	39.4 ±1.1 a	93 ±1 a	21.6 ±0.1 a
	Tsuruoka	635 ±33 b	485 ±23 b	65 ±2 a	31.4 ±0.6 ab	94 ±1 a	21.4 ±0.3 a
	Kiyosato	600 ±17 bc	458 ±32 bc	64 ±7 a	29.1 ±0.8 b	95 ±1 a	21.8 ±0.3 a
	Ohgata	598 ±44 bc	397 ±31 bc	74 ±3 a	29.4 ±1.4 b	94 ±0 a	21.4 ±0.1 a
	Naruko	492 ±34 cd	357 ±13 bc	73 ±4 a	26.1 ±0.4 b	93 ±0 a	21.7 ±0.5 a
	Shikama	549 ±24 bc	397 ±26 bc	66 ±2 a	26.1 ±0.9 bc	94 ±1 a	22.3 ±0.2 a
	Mohka	396 ±14 d	326 ±23 c	60 ±2 a	19.6 ±0.6 c	92 ±1 a	22.0 ±0.1 a

† Variation shows standard error (n=3).

‡ Means with different letters differ significantly among soils in each year and subsoil plot ($p<0.05$, Tukey-Kramer's method).

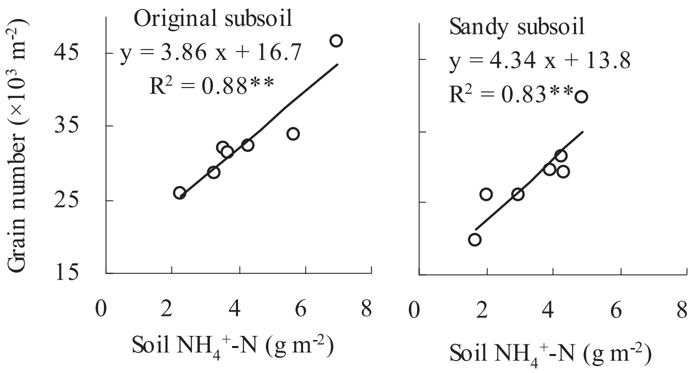

Fig. 5 The relationship between the amount of total soil NH_4^+-N in the plow layer at 40 DAT and grain number in 2005. ** means the relationship is significant ($p<0.01$).

tionship between ammonium-N in PL on June 10 and 20 and panicle number in Yamagata Prefecture, the Tohoku district of Japan. They considered that ammonium-N in the PL on June 10 showed the amount of N that could be absorbed by rice plant during the tillering stage.

7. Regression analysis to determine soil properties related to rice yield

In 2004, a multiple regression could not be applied because the available N and the ammonium adsorption of PL were significantly related to each other among the six soils. Table 4 shows results of regression analysis between the available N and the ammonium adsorption of PL and the soil ammonium-N at tillering stage (40 DAT), the grain number, the brown rice yield and the N uptake among the seven soils including Fujishima soil in the OS and SS plots in 2005. In the OS plots, the total soil ammonium-N at the tillering stage, the grain number, the brown rice yield and the N uptake at the harvest were effectively explained by the available N of PL. The ammonium adsorption of PL was not selected as an important parameter. It is reasonable that the ammonium adsorption of PL did not strongly affect the N uptake in the OS plots because leaching and denitrification of ammonium-N were not strongly promoted by percolation even in soils with the small ammonium adsorption of PL. The equation of the N uptake has a large intercept value (7.79 g m^{-2}) and shows that the contribution of the available N of PL to the N uptake was about 44 % at most (in Fujishima soil). The coefficient of determination in the equation of the N uptake was not large (R^2=0.58). The large intercept value and the small coefficient of determination were probably because of (1) the fertilizer N uptake (2.2-2.7 g m^{-2}), (2) uptake of N mineralized from the subsoil, (3) uptake of N fixed by bacteria from air and (4) added N interaction, for which uptake of soil N increased by application of readily available N fertil-

Table 4 Equations of the most effective regression by stepwise selection and their coefficients of determination (R^2) .

	regression	$R^{2\dagger}$
Original subsoil.		
Y, Soil NH_4^+-N in tillering stage.	Y= 0.55×(available N) +2.14	0.43
Y, Grain number	Y= 2.75×(available N) +22.5	0.73*
Y, Brown rice yield	Y= 57.1×(available N) +455	0.76*
Y, Nitrogen uptake at harvest	Y= 0.80×(available N) +7.79	0.58*
Sandy subsoil.		
Y, Soil NH_4^+-N in tillering stage.	Y= 1.13×(NH_4^+-N ad.) + 0.23×(available N) - 3.02	0.78*
Y, Grain number	Y= 2.53×(available N) +19.0	0.77*
Y, Brown rice yield	Y= 51.6×(available N) +383	0.72*
Y, Nitrogen uptake at harvest	Y= 0.64×(available N) +6.52	0.60*

† * means the regression was significant ($p<0.05$).

izer (Jenkinson et al., 1985). It is also considered that the available N of PL (evaluated by the anaerobic incubation using fresh moist soils in 30 ˚C for only 4 week) did not show certainly the amount of N mineralization during rice growing season.

In the SS plots, it was hypothesized that the effects of the ammonium adsorption of PL on the N uptake and the brown rice yield were clearly observed, because the percolation promoted leaching and the rice plants depended their N uptake on PL. The soil ammonium-N at the tillering stage was more effectively explained by multiple regression using the available N of PL and the ammonium adsorption of PL than by simple regression with each parameter. We consider that the ammonium adsorption of PL affected the soil ammonium-N at the tillering stage in the SS plots, because greater amount of ammonium-N was retained against leaching and denitrification in soils with the large ammonium adsorption of PL (ex. Kiyosato and Ohgata soils) than in the other soils, as shown in Figure 1. However, the grain number was efficiently explained by the available N of PL in the SS plots, and the ammonium adsorption of PL was not selected as an important parameter, although the ammonium-N at tillering stage was significantly correlated to the grain number (Figure 5). The brown rice yield and the N uptake were also effectively explained, not by the ammonium adsorption of PL, but by the available N of PL. The grain number is closely correlated to the N uptake until panicle heading (Wada, 1969). The N uptake until panicle heading would be affected not only by the soil ammonium-N at the tillering stage but also the mineralization of soil organic N after the tillering stage and the recovery rate of fertilizer N topdressed at panicle initiation stage. After maximum tillering stage, the amount of soil ammonium-N is extremely small because of rapid uptake by rice plants (Shoji & Mae, 1984); leaching loss of N is also considered to be extremely small. Therefore, we considered that the effects of the ammonium adsorption of PL did not significantly relate to the N uptake until the panicle heading stage. The lack of significant relationship between the ammonium adsorption of PL and the N uptake or the brown rice yield was because the total N uptake of rice can be greatly affected by mineralization of soil organic N after the tillering stage and the difference of ammonium adsorption of PL were small among the soils used in this study. However, the ammonium adsorption of PL affected the total soil ammonium-N at tillering stage, which correlated with the grain number. Therefore, it is considered that the ammonium adsorption of PL contributed to the large intercept value and the small coefficient of determination, especially in the SS plots. The ammonium adsorption of PL may affect the brown rice yield and the N uptake in sandy soils rich in kaolin minerals, in which percolation is extremely large and the ammonium adsorption of PL is very small.

Based on the above results in the field cultivation experiment using the seven soils (the available N of PL was 1.8-7.6 g m^{-2}; the ammonium adsorption of PL was 4.1-5.6 g m^{-2}), we concluded that the available N of PL significantly affected the N uptake and the brown rice yield. The effects of the ammonium adsorption of PL on the N uptake and the brown rice yield were not clearly observed by multiple regression analysis not only in the small percolation condition but also in the increased percolation condition by subsoil replacement with sand (average percolation rate 1-9 mm day^{-1}).

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Spectral detection of grazing degradation in the Xilingol Steppe, Inner Mongolia

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Abstract

In order to detect the grazing degradation of steppe vegetation, spectral reflectance was measured using a portable spectral radiometer in the Xilingol steppe, Inner Mongolia. There is only one main drinking place for animals, which is supplied by a water tank at the center of Branch No.4 site-00. In addition to the measurement of surface reflectance, vegetation surveys were carried out involving floristic composition and plant growth parameters as a function of the distance from site-00 at every 1-km interval until the 5-km point. It becomes clear that the effects of grazing on vegetation and soil mitigated according to the distance from site-00. Negative plant indicators of grazing intensity such as *Stipa grandis* and *Filifolium sibiricum* increased in dominance with distance from site-00, whereas positive indicators of grazing intensity, such as *Cleistogenens squarrosa* and *Agropyron cristatum*, decreased in dominance. Changes in growth parameters along the environmental gradients were reflected on NDVI (Normalized Difference Vegetation Index), in which NDVI was positively correlated with the aboveground biomass ($r = 0.77$, $P < 0.001$), plant height ($r = 0.69$, $P < 0.001$) and coverage ($r = 0.89$, $P < 0.001$), respectively. These results suggested that a spectral vegetation index is effective in the detection of the degradation of grazing grassland in a non-destruction way.

1. Introduction

Grasslands in China account for about 40% of the total territory of China, with more than 355 million ha in area (Ni, 2002). Inner Mongolia holds more than 22% of the total grassland areas of China, and the grassland has been used for grazing by nomadic people for thousands of years, and part of the grassland has been subjected to cultivation since the 1950s. Akiyama and Kawamura (2007) gave gross observation on grassland degradation in China. The Xilingol steppe is a major husbandry region in Inner Mongolia. However, a large area of the steppe is suffering from on-going desertification due to human activities such as overgrazing (Zhou et al., 1995; Li et al., 2000).

Many studies have been conducted concerning the effects of grazing on vegetation in the Xilingol Steppe. There are three types of steppes in Xilingol: meadow steppe, typical steppe and desert steppe. Typical steppe occupies most of the area. Meadow steppe is mainly dominated by *Stipa baicalensis*, *Filifolium sibiricum* and other forbs, whereas typical steppe can be grouped into *Leymus chinensis* steppe and *Stipa grandis* steppe (Li et al., 1988). Detecting the grazing intensity in a steppe for free grazing is the most difficult, and the distance from the nearest village is generally used as an indicator of grazing pressure (Nakamura et al., 2000). The line transect method and long-term point research are useful for investigating the impacts of grazing on vegetation, and some results have been achieved (Li, 1994, 1996; Li et al., 1994; Nakamura et al., 1998, 2000). *Leymus chinensis* and *Stipa grandis* steppes, which are the major original vegetation of typical steppes in Inner Mongolia, shift to an *Artemisia frigida* type steppe under long-term heavy grazing, and to a *Potentilla acaulis* type steppe under sustained overgrazing (Li, 1994, 1996; Li and Wang, 1999). However, steppes under moderate grazing achieved the highest species diversity and ANPP (aboveground net primary production), suggesting that a sustainable utilization of steppe is possible if it is kept under careful management (Chen and Wang, 2000; Li, 1994, 1996; Wang and Wang, 1999a; Wang et al. 1999a, 1999b).

On the other hand, trials for the measurement of bio-information using spectral radiometers had been attempted on many crops and pasture plants, and these results have been summarized by Akiyama (1996). Akiyama and Shibayama (1985) measured six pasture grass populations with three different cutting levels using a field-type radiometer which covers wavelengths of 400-1200nm. As the result of spectral analysis, 650, 850 and 1040 nm reflectance factors are the most suitable for estimating aboveground biomass, leaf area index and the amount of nitrogen in stands. In addition, Itano et al. (2000) measured an Italian ryegrass (*Lolium multiflorum* Lam.) population for obtaining information on pasture management. By observing reflectance intensities at 6 wavelengths ranging from within the visible region to the near-infrared, it was possible to acquire bio-information such as plant coverage, height, aboveground biomass and LAI. Lodging information was also detectable. Spectral reflectance measurement is a promising method for monitoring the condition of the grassland in a non-destructive way and one that is connected with satellite image analysis.

As satellites onboarding super-high resolution and hyper-spectral sensors like IKONOS or EO-1/Hyperion have become ready at hand in recent years (e.g. Thenkabail, 2003), the importance is increasing to collect the site specific information as of the ground truth data. This is the reason that we choose spectroradiometric method corresponding to field data.

Although some concepts and considerations have been given in relation to evaluating and monitoring the qualities of steppes (Li, 1994, 1996; Li and Wang, 1999; Nakamura et al., 2000), there is still the lack of a standard method for judging steppe degradation which is easy to employ for such a huge area. Therefore, the establishment of a management system for the sustainable utilization of grassland is urgently required. We chose an area specified by the Landsat/TM image in the Xilingol Steppe, where the distance from the village can be used as an effective indicator for grazing intensity since the water tank in the village (Branch No.4 of the Baiyinkulun Livestock Farm) is the main drinking place for animals. We therefore analyzed the relationships between the extent of grazing degradation and spectral reflectance factors as a function of the distance from the water tank and combined this with a vegetation survey. The purpose of this study was to test whether the spectral method is effective for detecting grassland degradation in a non-destructive way in the Inner Mongolia steppe.

2. Methods

2.1 Site description

2.1.1 Baiyinkulun Livestock Farm

The Xilingol steppe is situated in the northeastern part of Inner Mongolia, which is about 400 km north of Beijing. The Baiyinkulun Livestock Farm is one of the farms of the Xilingol League and is located in the southern Xilingol Steppe (Fig. 1). The area of the Baiyinkulun Livestock Farm extends approximately 50 km east to west and 30 km north to south.

The steppes are subjected to grazing or mowing spread around the surrounding small villages with water tanks to supply water for living and animal husbandry (Fig. 1). The Baiyinkulun Livestock Farm was an important state livestock farm used for raising military horses during the period of 1950 to 1970. Since the 1970s, it has become a local government-managed livestock farm, and occupies 141,650 ha of grassland in total of which 138,000 ha is used for livestock (grazing and mowing) and 3,650 ha for cropland.

2.1.2 Pipelines

Securing water for animals is the most serious problem which this district faces because the hard base rock prevents the digging of water wells. From 1965 to 1969, pipelines were constructed from the Liyupaozi Lake for transporting fresh water at a distance of 73.2 km. Here, in the Baiyinkulun Livestock Farm, 10 water tanks were built at 5 to 7 km intervals for supplying water for daily living as well as for animals (Fig. 1). Therefore, nomadic people built their houses near the tanks, forming small villages. As thousands of animals went to the tank and back every day to drink water, the land around the drinking place became bare. By carefully watching the summer

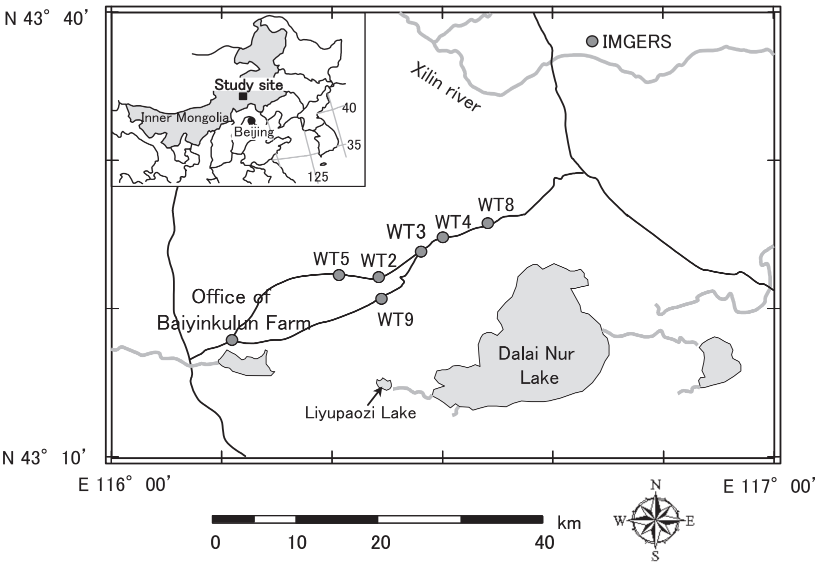

Fig.1. Location of Baiyinkulun Livestock Farm and water tanks along the pipeline, with an inset map showing the location of the study site in northern China.

satellite images of this area, we noticed bright spots appearing at intervals of several kilometers. At the center of each of these, there are circles of bare soil of over 100 m in diameter, which have been formed by the frequent trampling by domestic animals. The gradient of grazing pressure might mitigate in concentric circles inversely proportional to the distance from the village center.

2.1.3 Branch No.4 (Silian village)

The Baiyinkulun Livestock Farm consists of 10 branches. Among them, we are watching with interest Branch No.4 (villagers call it Silian) at water tank No.3 (WT-3). Most of the residents are raising sheep, cattle or goats. The altitude here ranges from 1300 to 1400 m, a gentle slope spreads to the north, and grasslands extend for more than 10 km in each direction. Besides this, several old craters of small volcanic mountains are situated here. Fig.2 is the study site displayed on a Landsat/ETM+ image acquired on August 14,

2001. The cross symbols (×) represent the sampling points for 1999. The total area of Branch No.4 is about 20,000 ha, of which 19,000 ha were available grasslands, consisting of 13,000 ha for grazing and 6,000 ha for mowing according to the statistics of 1997. No croplands nor protective fences for separating grazing from mowing grasslands in this Branch were observed during the experiment period (1998 to 1999). In 1973, Branch No. 4 consisted of only 35 families (172 people). However, this has since in-

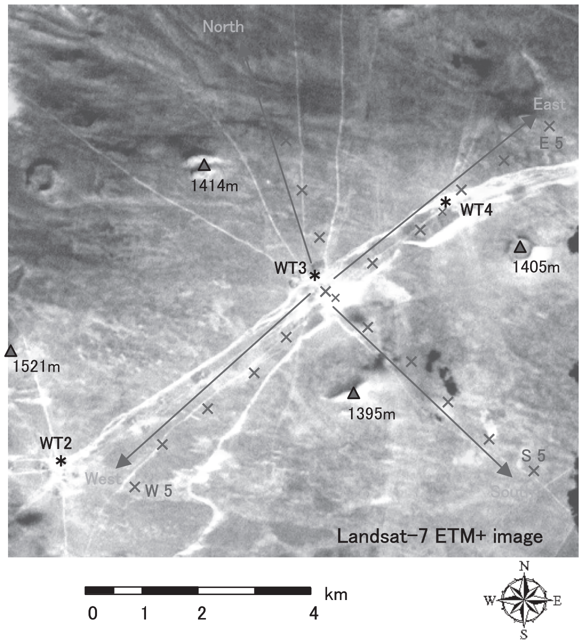

Fig.2. Setting of test sites for vegetation, soil and spectral measurements at Branch No.4 (WT-3) of the Baiyinkulun Livestock Farm in the Xiligol steppe, overlaid on Landsat/ETM+ image acquired on August 14, 2001. The red arrows show two courses from southwest to northeast (W-E course), and north to southeast (N-S course) for the field surveys.
△: Mountain, ＊: WT; Water tank, ×: Points for vegetation survey and spectral measurement; WT2: Water tank No.2 at power substation; WT3: Water tank No.3 at the center of Silian village (Branch No.4); WT4: Water tank No.4 at Silian village (about 2.5 km northeastern from center); Landsat-7 ETM+ satellite data acquired on August 14, 2001. (R:G:B = ETM5:ETM8:ETM2)

creased to 199 families (430 people) in 1999.

2.1.4 Increment of animal number

The yearly changes in animal numbers in Branch No.4 (Silian) are shown in Fig. 3. The horse was the major animal for the farm just after its foundation. However, this has since changed to sheep. The stock numbers increased steeply from years 1985 to 1987, partly due to the reorganization of branches. In addition, the increment in goat numbers is outstanding because of the soaring price of cashmere since 1995. Accordingly, the total livestock has increased to 35,600 HT (sheep equivalent animal unit) in 1999, where 1 horse is 6 HT, 1 cattle is 5 HT and 1 goat is 0.8 HT, according to Zhang and Liu (1992). Thus, the average grazing intensity, which is simply the ratio of the total livestock to grassland area, would be 2.74 HT ha^{-1} for grazing land or 1.89 HT ha^{-1} for the total grassland area, including the mowed land.

2.2 Setting of the test site

Branch No.4 (Silian village) is one of the villages located on the pipeline running east to west. WT-3 (water tank No. 3) at Branch No.4 is situated at 116 °28’10”E, 43°23’56”N. Along the pipeline, a local beaten road passing from southwest to northeast can be found; we call this a W-E course (Fig. 2). To the east from WT-3, WT-4 (water tank No. 4) and several farm houses are located between the 2.5-km (E2.5) and 3-km points (E3). WT-2 (water tank No. 2) and a village called Power substation is located at 6-km west along the pipeline. More than half of the villagers raise animals. On this road, large-sized motor-trucks come and go during the summer season for shipping out produced domestic animals and for carrying in wintering feed. A lot of nomadic people and animals go back and forth along the road. Meanwhile, in the north-south direction (N-S course), small local paths are running but it is difficult to pass full-sized trucks, so there is no farm house nearby.

In the four directions shown in Fig. 2, we used GPS equipment to decide on the test sites, as described below: site-00 was a drinking place near the WT-3. Twenty-one test sites were chosen every 1 km until the 5-km point from site-00 in 4 directions. For instance, at the 5-km point to the east, we called this site E5. Such a configuration in space is illustrated in Fig. 2. Addition to the 21 test sites, site E2.5 was chosen at a distance of 2.5 km from site-00, where there is another tank (WT-2). Besides, S0.1 was chosen at the edge of central circle of bare land, about 100-m south of site-00, to ascertain tolerant species under the highest grazing intensity. Vegetation survey was not able to conduct at site-00, while reflectance was not measured at E2.5 and N5 because of low solar radiation during the surveyed hours.

According to the climate record observed at the IMGERS (Inner Mongolia Grassland Ecosystem Research Station, Chinese Academy of Sciences) meteorological observing station, which is located 28-km north of Branch No.4 (Fig. 1), the annual mean precipitation is 340 mm, and the annual mean air temperature was 0.58oC from 1980 to 1998. The Penman arid index is 2.1 for the area (Li, 1996).

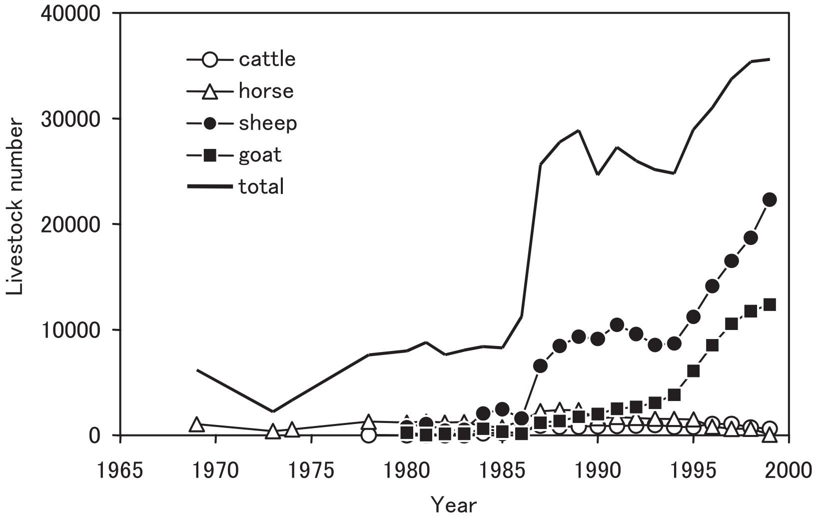

Fig. 3. Changes in animal numbers during the years of 1965 to 1999 at Branch No.4. Total numbers are calculated equivalent to sheep units (HT) where 1 horse is 6 HT, 1 cattle is 5 HT, 1 goat is 0.8 HT, according to Zhang & Liu (1992).

The steppe type in this area is typical steppe and meadow steppe (Li et al., 1988). Typical steppes dominated by *Stipa grandis* and *Leymus sinensis* are mainly present around the areas where the pipeline was established. Most of the typical steppes have been degraded to various degrees after being subjected to grazing for more than 40 years after the establishment of the livestock farm. Meadow steppes are present about 4 or 5 km north or south from the village towards the hills or lake. Accordingly, the soil types of this area are Chernozem for the meadow steppe and Chestnut for the typical steppe (Chen and Huang, 1988; XLSFWS, 1991).

Soil and vegetation surveys as well as spectral reflectance measurements were carried out during the summers of 1998 and 1999 around Branch No.4.

2.3 Vegetation survey

The vegetation survey was conducted according to the method proposed by Braun-Blanquet (1964). Five 1-m^2 quadrates were examined at each site. Firstly, a central quadrat (Qc) was chosen, then another 4 quadrates (Qe, Qw, Qs, Qn) chosen that were stationed 10 m away from Qc in 4 directions (east, west, south and north, respectively) determined using a compass. The canopy height (cm) and total coverage (%) of each quadrat were then recorded, and the mean values of the five quadrates were used to represent the plant height and plant coverage of the site. In addition, within the central quadrat (Qc), the coverage (C, %) and height (H, cm) of each species was estimated. The species frequency (F, %) was measured using the point method of multiple contacts (100 points along two 20-meter lines across the plot, i.e. from Qe to Qw and from Qs to Qn), where F (%) is the number of species touched among the 100 points (Goldall, 1952). We used SDR (the summed dominance ratio) to indicate the dominance of the species in the community (Numata and Yoda, 1957). The species dominance (SDR) was then calculated using the equation: SDR = (H'+C'+F')/3, where H' is the relative plant height (cm), C' is the relative coverage (%) and F' is the relative frequency (%); H' (C' or F') = H (C or F) of the species/maximum value of H (C or F) in the quadrat, respectively. Due to the frequency of animal foraging and trampling, there appeared a 100-m circle of bare soil around the site-00. For the vegetation survey, S0.1 was therefore used instead of site-00, 100-m south of site-00, which was able to grow some plants in an extremely sparse coverage and low canopy height. All of the plants were named according to Liu and Liu (1988).

After the vegetation survey and spectral measurements, all aboveground parts were removed at the soil surface level, and the total fresh weight (FW, g) in situ was measured using a portable electric balance (Model-BL2200H, Shimadzu Co. Ltd., Kyoto, Japan). Parts of the samples were brought back to IMGERS to be oven dried at 80°C for the estimation of their dry weight (DW, g) of the biomass. A survey of species composition was carried out in 1998, and other quantitative information on the plant community was measured during the summer seasons of 1998 and 1999.

2.4 Soil hardness measurement

The soil hardness (SH, mm) was measured at nine points for each site using a Yamanaka standard type soil hardness tester (Fujiwara Scientific CO.LTD., Tokyo) after the removal of aboveground plant materials. The surface color and moisture of the soil were also measured, but it was too difficult to relate this to the land characteristics because they were strongly affected by the previous rainfall event.

2.5 Spectral reflectance measurement

In order to detect the grazing degradation of grasslands by a non-destructive method, spectral reflectance measurements were carried out. A field-type spectral radiometer (MD-01 type) was used in this experiment which was manufactured by PREDE CO.LTD., Tokyo. This device is able to measure reflectance at 4 visible (450, 545, 650, 699 nm) and 2 near-infrared wavelengths (750 and 850 nm). The measurer stands facing the sun, stretching the optical fiber over the plant canopy keeping a distance of about 1 m between the tip of the fiber and the canopy. As the instant view angle of this equipment is 10°, if keeping a 1-m distance, the reflectance value inside 35.3 cm in diameter can be measured. Measurements were carried out during clear daytime between 9:00 and 15:00 local time to get strong and constant solar radiation. The spectral reflectance was corrected to a relative value using a standard white board (Spectralon, Labsphere CO.LTD. USA). The spectral measurements were repeated 7 times for each quadrat. After omitting the maximum and minimum values, the remaining 5 data points were averaged. The measurements were repeated for all 5 of the quadrates for each site.

In order to compare the quantitative biological information of grasslands, we used NDVI, a spectral vegetation index. The NDVI (Normalized Difference Vegetation Index) is a popular spectral vegetation index proposed by Rouse et al. (1974). It comes from the principle that chlorophyll in green leaf absorbs red light, while mesophyll cells reflect the near infrared wavelengths. Therefore, it relates to the biomass and vigor of vegetation. The following is the equation for NDVI.

NDVI = (NIR - Red) / (NIR + Red)

Here, NIR is the intensity of near infrared reflectance at 850 nm, and Red means red reflectance at a wavelength of 650 nm in this experiment.

3. Results

3.1 Changes in species composition with distance from water tank

Fig. 4 shows the changes in dominance (SDR, %) of major species with increasing distance from site-00 in the N-S and W-E directions (see Fig. 2). As described above, site-00 is the drinking place for animals near the water tank No.3 (WT-3) of the Silian village (Branch No. 4). Due to frequent animal foraging and trampling, site-00 displayed a bare cover of plants. We illustrated the result of S0.1 in Fig. 4 to indicate vegetation under an extremely high grazing pressure 100 m from site-00 in a region where some plants are able to grow in an extremely low coverage of 20% and a plant height of 3.4 cm (also see Fig. 5a). With increasing distance from site-00 in all 4 directions (N, S, W, E), there was a similar tendency in terms of species dominance changes with distance

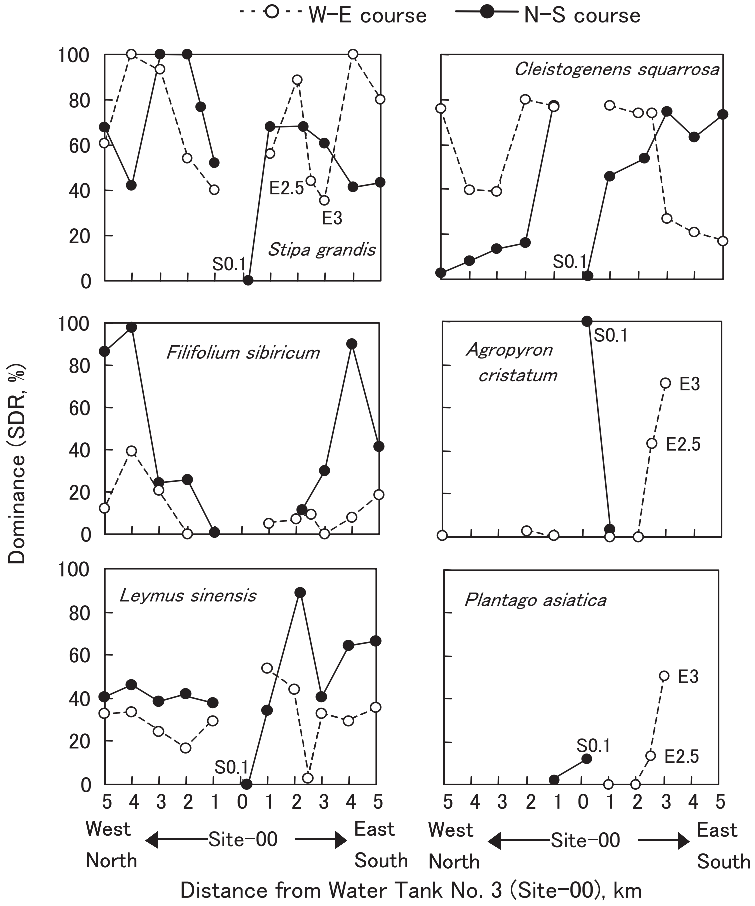

Fig. 4. Changes in dominance (SDR, %) of the major species with distance from the village of Branch No.4 (WT-3: site-00) in the Xilingol steppe (July, 1999). The W-E course is from site-00 to the southwestern and northeastern 5-km points, and the N-S course is from site-00 to the northern and southeastern 5-km points (detailed see Fig. 2). Inserted E2.5 and E3 indicates that these two sites are kind of special due to the fact that they are near WT-4 (water tank No. 4). Site-00 is bare land, therefore the results of S0.1, 100-m south of site-00, is illustrated to represent vegetation under extreme grazing pressures.

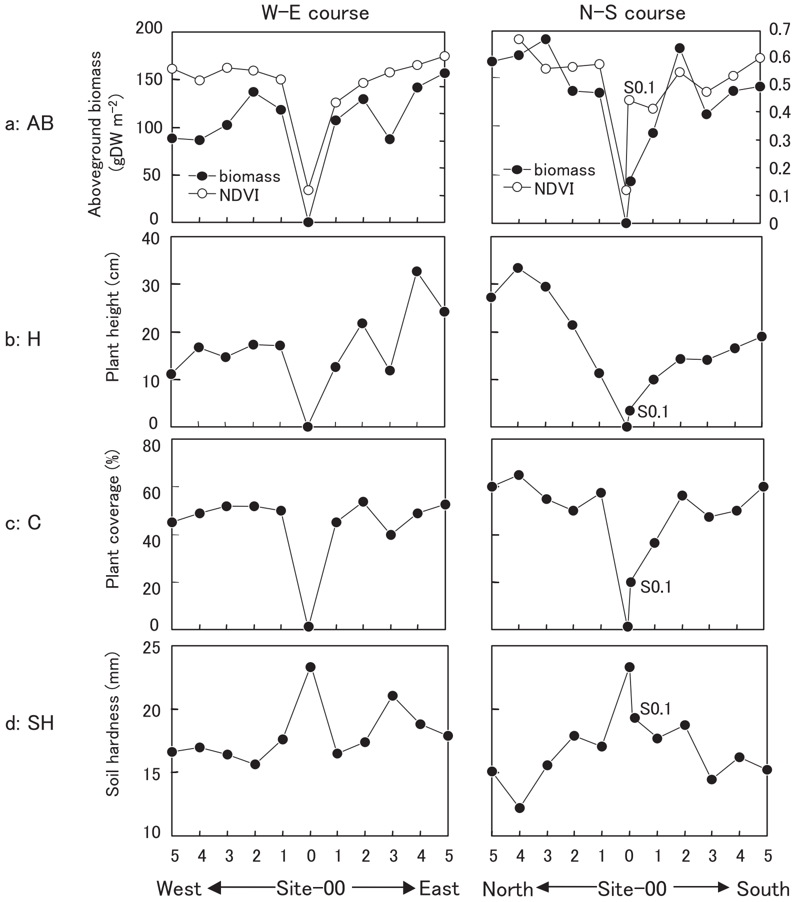

Fig. 5. Changes in plant quantity information and soil hardness according to the distance from the water tank at Branch No.4 of the Baiyinkulun Livestock Farm.
a: aboveground biomass (AB) and NDVI, b: plant height (H), c: plant coverage (C) and d: soil hardness (SH). NDVI is the Normalized Difference Vegetation Index obtained from the following equation: NDVI = (NIR-R)/(NIR+R), where NIR is the near infrared reflectance and R is the red one. NDVI at E2.5 and N5 were not measured because of the low solar radiation during surveyed hours.

from site-00. That is, negative indicators of grazing intensity suggested by Li et al. (1999) (see discussion for details), such as *Stipa grandis* and *Filifolium sibiricum*, increased in dominance (SDR, %) with distance from site-00. Whereas positive indicators of grazing intensity, such as *Cleistogenens squarrosa* and *Agropyron cristatum*, decreased in dominance with distance from site-00. Such a tendency in the W-E direction seemed not as clear as in the N-S direction as a result of disturbances due to the existence of other water tanks (WT-2 and WT-4) and farm houses along the pipeline. The areas inside a 1-km diameter from site-00 were thought to be subject to heavy grazing, where positive indicators for grazing intensity, i.e. *A. cristatum* and *C. squarrosa*, dominated the communities. Whereas negative indicators for grazing intensity, i.e. *S. grandis* and *F. sibiricum*, were absent or with lower SDR values at sites a distance of 1 km from site-00. In the meantime, a widespread weedy species, *Plantago asiatica*, appeared at these sites, also indicating the effects of human daily life. Because of another water tank (WT-4) located near E3, sites E2.5 and E3 exhibited a reduced SDR of *S. grandis* and an increased SDR of *A. cristatum*, as well as the presence of the weedy species *P. asiatica*.

Similarly, the grazing pressure seemed to decline with distances of greater than 2 km from site-00, since the biomass increased from 42.9 g DW m^{-2} (S0.1 at 100 m away from site-00) to more than 180 g DW m^{-2} (see Fig. 5a). For sites at distances of between 2 and 3 km away from site-00, *Stipa grandis* and *Leymus chinensis* were found, and dominated the community with increasing SDR with distance away from site-00. Meanwhile, *Cleistogenes squarrosa* decreased in SDR with increasing distance away from site-00 for distances of more than 2 km, and this also was indicative of a decreased grazing intensity, especially for distances greater than 2 km away from the village.

For distances of 4 or 5 km from the village in the N-S direction, meadow steppe appeared instead of typical steppe. This area was mainly used for mowing or for light grazing by horses. *F. sibiricum* and *S. grandis* (data mixed with *Stipa baicalensis*) dominated the communities and with more richness in species, including many forbs.

3.2 Changes in quantitative biological parameters with distance from water tank

Fig. 5 shows quantitative biological information on the grasslands such as the aboveground biomass (a: AB), the plant height (b: H), the plant coverage (c: C) and the soil hardness (d: SH) as a function of distance from WT-3 (site-00) at Branch No.4 to the 5-km points for the four directions. Because there was a similar trend in biomass changed with a distance from site-00 towards to the four directions between 1998 and 1999, we only illustrated the results of 1999 in Fig. 5 for explanation.

In the N-S direction of Fig. 5a, AB increased linearly with increasing distance from site-00, and remained at over 160 g DW m-2 for distances of between 3 and 5 km (N3, N4 and N5). A similar trend is observed in the W-E direction, however, for the same reason as the changes in the species composition described above, it is not as clear as in the case of the N-S direction because of disturbances by the existence of other water tanks and farm houses along the pipeline. On the same graph (Fig. 5a), the changes in the NDVI values are drawn. The NDVI curves appeared to be almost proportional to AB, C and H.

Slow but constant increments were observed from site-00 to N4 in H of Fig. 5b and reached a maximum value (34 cm) at N4 in the N-S direction. In the W-E direction, the maximum value reached was 33 cm at E4, but the curve had roughness and hollows.

The coverage (C) in Fig. 5c showed almost the same patterns for the N-S and E-W directions. In both directions, steep V shapes formed between site-00 at the 1-km points, securing 50% coverage, then it became almost constant out to the 5-km points. The maximum coverage was 66% at N4. Hollows at E3 were commonly found in all three sets of data. This result might have been affected by farm houses near WT-4.

3.3 Soil hardness (SH)

The changes in SH are shown in Fig. 5d in relation to the distance from site-00. The values are over 20 mm (very tough) around site-00, but soften with distance to 12 mm at N4. This trend was clear in the northerly direction, but was obscure in the W-E direction. The SH curve had another peak at the E3 site.

4. Discussion

4.1 Floristic composition changes after long-term heavy grazing

Li et al. (1999) established a grazing experimental plot (21 ha) with 21 subplots in IMGERS and compared it with different stocking rates in an *Artemisia frigida* steppe about 30-km north of Branch No. 4 in the Xilingol steppe. This study began in 1990 with the aim of investigating short and long-term effects of grazing on vegetation and soils. The results of the first 3-5 years have been reported involving changes in species composition, species diversity, as well as the above and belowground biomass with grazing management (Han et al., 1999; Li and Wang, 1999; Wang and Wang, 1999a, 1999b). Li (1994) suggested six ecological groups in relation to grazing intensity in the steppe, *i.e.* decreaser, disappearer, increaser, invader, species adapting to moderate grazing and fluctuating species. Decreaser and disappearer are negative indicators for grazing pressure such as *Achnatherum sibiricum*, *Stipa grandis* and *Filifolium sibiricum*, while increaser and invader are positive indicators such as *Carex duriuscula*, *Artemisia frigida*, *Cleistogenes squarrosa*, *Agropyron cristatum* and *Potentilla acaulis* (Li, 1994; Nakamura et al., 1998; Han et al., 1999).

In this study, such a change in species composition with distance from the village was clear in the N-S direction, but was disturbed by the existence of farm houses along the W-E direction. For example, due to the WT-4 located at 2.7-km east of the Silian village, these sites (E3 and E2.5) exhibited a reduced dominance of *Stipa grandis* and an increased dominance of *Agropyron cristatum*, as well as the appearance of the weedy species *Plantago asiatica*. A similar tendency was observed at the W4 and W5 sites close to WT-2 (Fig. 4). However, along the N-S direction, there were no farm houses found in the areas at distances of between 1 km and 5 km from the site-00. It was obvious that the changes in species composition along the N-S direction could be explained by long-term grazing effects with decreasing grazing intensity with increasing distance away from site-00. Moreover, it indicated that areas at a distance of 3 km away from the village (i.e. at S3 or N3) may be under a relatively moderate grazing pressure, reaching higher biomass and dominated with *Stipa grandis*, with the highest SDR in the N-S direction (Figs. 4 and 5a).

4.2 Relations between NDVI and biological parameters

Fig. 6 shows the relationships between NDVI and several biological parameters, such as the aboveground biomass (AB), the coverage (C), the plant height (H) and one soil parameter (soil hardness, SH) measured during the 1999 experiment using the same data as in Fig. 5.

This includes all sites on the W-E and N-S courses. A high correlation was observed, particularly between NDVI and C ($r = 0.89$, $P < 0.001$, $n = 21$). The correlations of NDVI vs. AB ($r = 0.77$, $P < 0.001$, $n = 21$), and NDVI vs. H ($r = 0.69$, $P < 0.001$, $n = 21$) were also significant, although the correlation coefficient was not as high as that of NDVI vs. C. The same tendency was reported using satellite data analysis (Kawamura et al., 2003), that the correlation was much higher in the coverage (%) than in plant height because remote sensors sharply sense the percentage plant coverage in the nadir angle, so it is more sensitive to horizontal information than to vertical. Xiao et al. (1997) analyzed Landsat/TM data for detecting steppe vegetation types in Xilingol using 1987 image. In the multispectral classification, 14 vegetation cover types were recognized. Among them, the total area of degraded and desertificated grasslands, summed from the *Stipa krylovii* steppe, *Artemisia frigida* steppe and desertificated grasslands, accounted for 26.4% of the total 29,440 km^2 of land area.

4.3 Relation of spectral reflectance and grazing degradation

Land degradation triggered in arid/semiarid grasslands in the world is mainly caused by overgrazing (UNEP, 1984). However, by raising freely several thousands of flock on several thousands of hectares of grassland, it is difficult to quantify grazing pressure in each area. Without such a grassland management record, how can we judge objectively the extent of the overgrazing? A simple ratio of the total animal

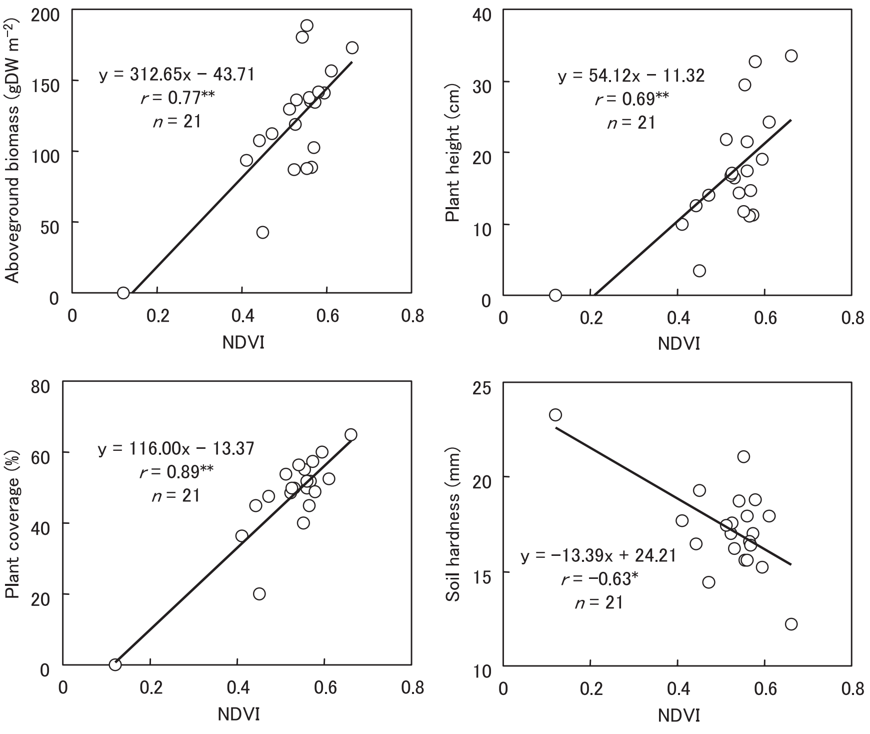

Fig. 6. Relationships between NDVI and several plant quantity parameters observed in 1999 at Branch No.4. Each point coordinate data used 21 test sites in Fig.5.
Upper left; aboveground biomass (AB) vs. NDVI; Upper right: plant height (H) vs. NDVI; Bottom left; plant coverage (C) vs. NDVI; and Bottom right; soil hardness (SH) vs. NDVI
* $P < 0.01$; ** $P < 0.001$.

numbers 35,600 HT by the grassland area yields 2.74 head ha^{-1} in average over 13,000 ha at Branch No.4. Although it is similar to the optimal stocking rate (2.67 head ha^{-1}) obtained by the grazing experiments done by Wang et al. (1999a) at IMGERS, the area around the village is suffering from overgrazing of hundreds of times this level.

In semi-arid districts, a deterioration of grassland is easily brought about by overgrazing, meaning a frequent removal of herbage. In addition, the treading by animal causes a destruction of aboveground organs and root systems as a result of soil compaction (Kubo and Akiyama, 1977).

The possible mechanisms of grazing degradation are summarized in Table 1. Herbage biomass decreases and plants become dwarfed, and plant litters are removed by grazing animals and strong wind. Herbage production reduces by the serious and continuous removal of assimilation organs under ceaseless heavy grazing, sometimes resulting in bare land. This is why it is easy to dry up the soil surface. At the same time, the floristic composition become poor and simple, but some tolerant species prosper forming rugged patches consisting of unpalatable species. Meanwhile, the soil becomes compact by frequent trampling, and defecation brings about an enrichment of the soil chemical components by supplying large amounts of feces and urine exhausted by animals. At the ecosystem level, the soil surface becomes dry, resulting in a decline in herbage productivity and species diversity. Most of these changes in plant and soil conditions are related to an enhancement in the brightness captured by spectral-radiometer or satellite sensors which are reflected in NDVI values in this experiment. These are the characteristics of the direct deterioration of grassland caused by overgrazing.

The extent of land degradation occurred so rapidly around the village, that the biomass and the floristic composition had changed drastically step by step. Landsat/TM data of middle-resolution can not follow such sudden changes. In that meaning, getting the field spectral data together with the biological information are useful for using super-high resolution satellite data in the near future. Under similar point of view, Harris and Asner (in print) detected grazing gradient with airborne imaging spectroscopy in Utah, USA, and showed that detection of persistent grazing gradients with imaging spectroscopy is feasible. But they did not refer about changes in the floristic composition.

The spectral reflectance data could not detect the changes in floristic composition directly in this experiment. Fukuo et al. (2000) measured some unpalatable species communities such as *Iris*, *Potentilla* and *Caragana microphylla* using the same spectroradiometer of this study, and found that there existed weak relationships between vegetation indices and plant coverage ratio of the species. However, it was difficult to generalize for such unpalatable communities. In the meanwhile, inedible species for animals are apt to form distinctive and pure communities near the village, which may be detectable from the difference of texture in the highly resolution satellite images. By this method, Yamamoto (personal communication, 2000) detected weed communities in grasslands using roughness index of satellite image. If we can find a spectral index able to specify plant indicators for grazing degradation, spectral detection of grazing degradation may improve to detect the changes in floristic composition due to overgrazing. Nevertheless, our results indicated that NDVI achieved by spectral reference could well reflect the

Table 1. Effects and mechanisms of land degradation caused by overgrazing and its detection using spectral reflectance

Animal actions	Effects on plant & soil	Changes of plant & soil	Ecosystem level
Foraging	Plant height & biomass	Dwarf and bare*	Decline in productivity
Foraging	Litter accumulation	Dissappear*	Soil drying
Foraging	Composition	Deforage tolerant species	Floristic deterioration
Foraging	Unpalatable community	Rugged surface	Floristic deterioration
Trampling	Lodging and organ dying	Loss of leaf area*	Decline in productivity
Trampling	Soil (physical effect)	Soil harden*	Soil degradation
Defecation	Soil (chemical effect)	Enrichment	Weedy species

*: Increase reflectance or decrease NDVI.

growth parameters such as aboveground biomass, plant coverage and height along grazing intensity gradients. This strongly suggested that spectral detection of grazing degradation with a spectral vegetation index (e.g. NDVI) is effective in the Xilingol steppe in a non-destruction way.

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Metal complexation of humus and horizon differentiation in Andisols and Spodosols

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Abstract

From tephra, not only Andisols, but Spodosols can also be formed depending on changes in the soil formation factors. In Andisols, the *in situ* accumulation of humus in the A horizon and that of the active Al and Fe in the A and Bw horizons are the major soil formation processes, whereas in Spodosols, the eluviation and illuviation of organic matter (OM), Al and Fe are predominant. Among the soil characterization data, the pyrophosphate-extractable Al (Al_p), cation exchange capacity (CEC) and organic carbon (OC) content were selected in order to examine the similarities and differences in metal complexation and related properties of these soils. The A horizon soils of the Andisols and the Bh-Bhs and Bs horizon soils of the Spodosols derived from tephra (SPT) and those derived from nontephra deposits (SPN) are similar in their Al_p/OC ratios suggesting that the metal complexation of humus is a property common to these horizons. In contrast, the Al_p/OC ratio of the E horizon soils in the SPT and SPN was significantly lower than those in the Bh-Bhs and Bs horizon soils of the SPT and SPN and the A and Bw horizon soils of the Andisols. Furthermore, the CEC in these E horizon soils did not exceed those in the other horizon soils. These results indicate that the static OM in the E horizons has less total carboxyl groups, including both the complexed and free ones, than that in the other horizons. Recent information on the SPN formation was applicable to the SPT according to their soil characterization data.

Introduction

Metal complexation is one of the factors affecting the stabilization of organic matter (OM) in soil (Wada and Higashi, 1976; Shoji et al., 1993; Kögel-Knabner et al., 2008). The stabilization of OM contributes to sequestrating C in soil. Nutrient release through mineralization of OM for plants and microorganisms in the soil is also affected by metal complexation (Olk 2006; Olk and Gregorich, 2006). Soluble OM enhances translocation of the metallic elements in the soil whereas insoluble OM fixes heavy metals in the soil. Polyvalent cations are effective in precipitating OM (Adachi, 2003). Other important roles of OM in the soil are the sorption of hydrophobic organic chemicals, keeping the soil's physical, chemical and biological properties in good conditions, etc.

In Andisols (AND), abundant humus is accumulated in their A horizons (Fig. 1) and is mostly immobile (Torn et al., 1997; Huang, 2004) at least for the past thousands of years as demonstrated by multi-sequal soil profiles. One of the interpretations for the high stability of humus in AND is the complexation with Al (Wada and Higashi, 1976). The approximate chemical formula of the Al-humus complex in the A horizon of AND was proposed to be $\{C_{36}(COO[Al])_3COO^-\}_n$ (Nanzyo and Shoji, 1993).

Under humid and appropriately cold soil environments, functions of the dissolved organic materials (DOM) that are biologically produced are strongly expressed and Spodosols (Fig. 1) are formed (van Breemen et al., 2000). Spodosols show a strong horizon differentiation resulting from the translocation of the humus, Al and Fe from the upper horizons and their accumulation in the underlying spodic horizons (Lundström et al., 2000a). Detailed studies were carried out to understand the Spodosol formation processes in Nordic countries (Lundström et al., 2000b). Although it is common that AND form on tephra de-

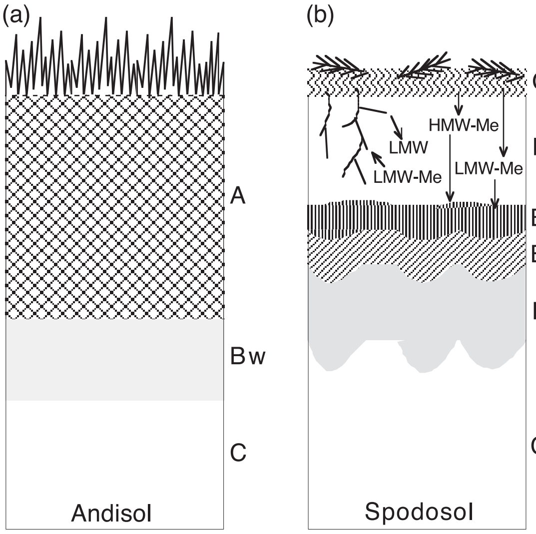

Fig. 1. Schematic diagram showing profiles of Andisol (a) and Spodosol (b). LMW represents low molecular weight organics of less than 1 kD and HMW represents high molecular weight organics of 1 kD or more. Mycorrhizal fungi releases LMW, dissolves metallic ions (Me) and translocates LMW-Me complexes (Lundström et al., 2000b).

posits in a humid climate, Spodosols can also form on tephra during cold and humid climatic conditions. Ito (1994) described the characteristics of tephra-derived Spodosols (SPT) as a high OM content, frequent occurrence of Humods and occurrence of one or more E horizons in a pedon. The E horizon soils of SPT contain scarce allophane and tend to be thinner (about 5 cm) and darker than those of Spodosols derived from nontephra deposits (SPN).

The objectives of the present study were (i) to compare the degree of complexation between the metal and humus in different soil horizons of AND, SPT and SPN, and (ii) to discuss the differences in properties among these soil horizons based on recent researches (Lundström, et al., 2000b; Kögel-Knabner et al., 2008; von Lützow et al., 2008).

Materials and methods

Several sets of soil characterization data were used to compare the properties of 8 horizon soils that are A and Bw horizon soils of AND, and the E, Bh-Bhs and Bs horizon soils of SPT and SPN. They are from the Andisols TU database (Shoji et al., 1993), a database by the Kurobokudo Cooperative Research Group (Wada, 1986), the tour guide of the Ninth International Soil Classification Workshop (SMSS and Japanese Committee, 1987), the database on SPT (Ito, 1994), the tour guide of the Fifth International Soil Correlation Meeting (SCS-USDA, 1988a), the narrative pedon description and primary characterization data of Alaska soils (SCS-USDA, 1988b), and three Spodosols from Nordic countries (Karltun et al., 2000).

Based on these data sets, the soil horizon data were classified into 8 groups according to their soil order, parent materials and horizon designation as shown in Table 1. They are the A and Bw horizon soils of AND, and the E, Bh-Bhs and Bs horizons soils of SPT and SPN. These data include buried horizons but the uppermost A horizons were excluded from the A horizon soils of AND in order to reduce the effect of fresh OM. The Bh and Bhs horizons were combined in one group as Bh-Bhs. The uppermost Bs horizon soil was used from the 3 Nordic pedons as each Bs horizon had several subdivisions (Karltun et al., 2000). In order to examine the metal complexation of OM, the OC content, pyophosphate-extractable Al (Al_p) and Fe (Fe_p) were obtained from these data sets. The OC content in the fine earth fraction of the air-dried soils was determined by the dichromate method (Ping et al., 1989) or the dry combustion method (Shoji et al., 1993). Al complexed with humus is best evaluated as Al_p (Dahlgren, 1994). In addition to Al_p, Fe_p was also used to evaluate the Fe complexed with humus in the SPT and SPN although oxalate-extractable Fe is mostly ferrihydrite in the AND (Child, 1991). Logarithmic class intervals were used for the histogram construction to cover the widespread data. In order to examine the relationships between the OC and cation exchange capacity (CEC), the soil horizon data were grouped into 15 classes according to the index cation used to determine the CEC, soil orders, parent materials and horizon designation as shown in Table 2.

Results and Discussion

1. Frequency distribution of OC content

Accumulation of abundant OC ranging between 10 and 200 g C kg^{-1} is an outstanding characteristic of the A horizons in AND. A similar amount of OC (40 - 230 g C kg^{-1}) is also accumulated in the Bh-Bhs horizons of SPT as shown in Fig. 2. A large amount of OC is also accumulated in the Bh-Bhs horizons of SPN although it is less than that in SPT. Thus, all of these horizons show high OC contents in spite of the fact that their genetic processes are not the same.

Table 1. Collected soil horizon data to examine metal complexation.

Soil order	Locations of pedons	Horizon designation[*1]	Number of pedons	Number of horizons	References
Andisols	Alaska, Northeastern Japan	A[*2]	33	85	Shoji et al. (1993)
		Bw	33	61	
Spodosols (formed on tephra)	Alaska, Northeastern Japan	E	37	48	Ito (1994), Shoji et al. (1993), Shoji and Yamada (1991)
		Bh, Bhs	30	52	
		Bs	36	64	
Spodosols (formed on nontephra)	New England, Nordic countries	E	8	9	Shoji and Yamada (1991), SCS-USDA (1988a,b), Karltun et al. (2000)
		Bh, Bhs	5	8	
		Bs	10	17	

[*1]: Buried horizons were included.

[*2]: Uppermost A horizons were excluded to reduce the effect of fresh plant residues.

Table 2. Soil hoizon data collected for examining relationships between OC and CEC.

Index cation	Soil order	Locations of pedons	Horizon[*1] designation	Number of pedons	Number of horizons	References
NH_4^+	Andisols	Kanto to Hokkaido	A[*2]	13	34	SMSS-USDA and Jpn Committee (1987)
			Bw	12	25	
	Andisols	Kyushu to Hokkaido	A[*2]	25	77	Wada, 1986
			Bw	18	33	
	Spodosols (formed on tephra)	Alaska, Northeastern Japan	E	21	33	Ito (1994), Shoji et al. (1993), Shoji and Yamada (1991)
			Bh, Bhs	19	32	
			Bs	23	51	
	Spodosols (formed on nontephra)	New England, Nordic countries	E	8	8	Shoji and Yamada (1991), SCS-USDA (1988a,b), Karltun et al. (2000)
			Bh, Bhs	7	9	
			Bs	7	16	
Ca^{2+}	Andisols	Alaska, Northeastern Japan	A[*2]	33	85	Shoji et al. (1993)
			Bw	33	61	
	Spodosols (formed on tephra)	Alaska, Northeastern Japan	E	8	14	Shoji et al. (1993)
			Bh, Bhs	3	8	
			Bs	9	14	

[*1]: Buried horizons were included.

[*2]: Uppermost A horizons were excluded to reduce the effect of fresh plant residues.

OM in the A horizons of AND accumulates *in situ* whereas OM in the Bh-Bhs horizons of SPN translocate from the overlying horizons. OM in the Bh-Bhs horizons of SPT may partly be a combination of OM translocated from overlying horizons and that previously accumulated in the buried soil because SPT has sometimes two or more E horizons.

The OC contents in the Bs horizons of SPT and SPN are lower than those in the Bh-Bhs horizons (Fig. 2) and the OC content in the Bh-Bhs and Bs horizons of SPN tends to be lower than that of SPT. The OC content in the Bw horizons of AND tend to be even lower than those in the Bs horizons of both SPT and SPN. Thus, translocation of OM from the A to Bw horizons, if any, is less than the OM illuviation in the Bs horizons of SPT and SPG.

The biggest difference in the OC content was found in the E horizons between SPT and SPN. The OC

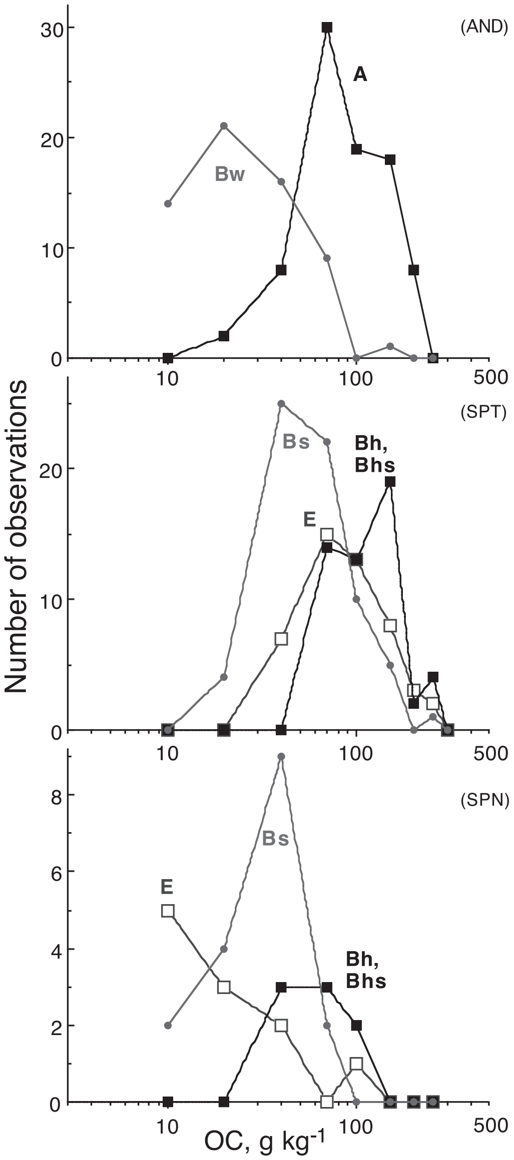

Fig. 2. Frequency distribution of organic C (OC) content in the A and Bw horizon of Andisols (AND), E, Bh-Bhs and Bs horizon of Spodosols derived tephra (SPT) and those derived from nontephra (SPN). For example, a plot at 20 g kg^{-1} shows the number of observations for $10 < \mathrm{OC} \leqq 20$ g kg^{-1}.

content in the E horizons of SPT is much higher than that of SPN as Ito (1994) stated. The OC content in the E horizon of SPT is similar to that in the Bh-Bhs horizons of SPT and also to that in the A horizons of AND. The total dissolved OC content of the 3 SPN pedons in Nordic countries (van Hees, et al., 2000) ranged between 4 and 9 mmol L^{-1}. As the content of the readily mobile OC is calculated to be 0.018 and 0.078 g kg^{-1}, postulating a water content is 500 g kg^{-1}, the OC in the E horizons is mostly static.

2. Relationship between OC and Al$_p$

The Al$_p$ content shows Al complexed with humus although the content is changeable with pH (Wada and Okamura, 1980; Takahashi et al., 2006). A linear relationship between the OC and Al$_p$ was recognized for the A horizon soils of AND (Fig. 3) as previously reported by Wada and Higashi (1976) and Shoji et al. (1993). However, the same plots for the SPT (Fig. 4) and SPN (Fig. 5) were more scattered than those for AND in spite of the fact that the O horizons were excluded from these plots. Among the plots for E, the Bh-Bhs and Bs of SPT and SPN, the plots for the Bs horizon soils are relatively linear distributed. In contrast, the plots for the E and Bh-Bhs horizon soils showed broad scattering and those for the E horizon soils of both SPT and SPN were in the low Al$_p$ area (Figs. 4 and 5, Ping et al., 1989; Shoji and Yamada, 1991). The plots for the Bh-Bhs horizon soils were distributed in the wide range between those for the Bs and E horizon soils. It was suggested from Figs. 4 and 5 that there is an upper limit in the Al$_p$/OC ratio.

In order to compare the degree of Al complexation in OM among the 8 groups of soils (E, Bh-Bhs and Bs horizons of SPT and SPG and A and Bw horizons of AND), the frequency distribution of the atomic Al$_p$/OC ratio (FD-Al$_p$/OC) of these soil horizons are shown in Fig. 6. A multiple comparison was done at the p=0.05 level according to Scheffé's F test after the logarithmic transformation. The Turkey-Kramer test was not used because the variance was not the same and the normality was abandoned in the FD-Al$_p$/OC

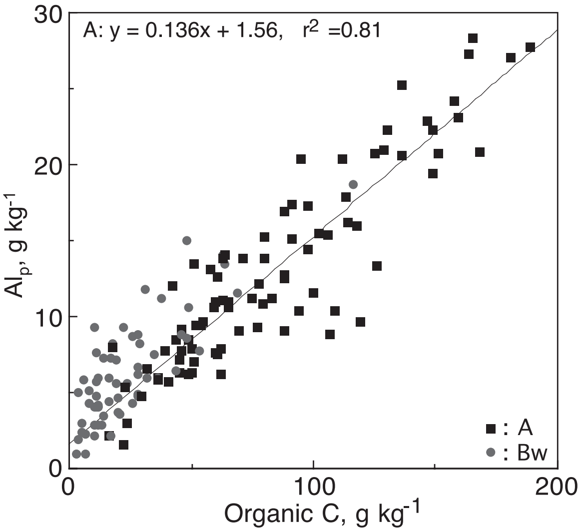

Fig. 3. Relationship between organic C content and pyrophosphate-extractable Al (Al$_p$) of Andisols.

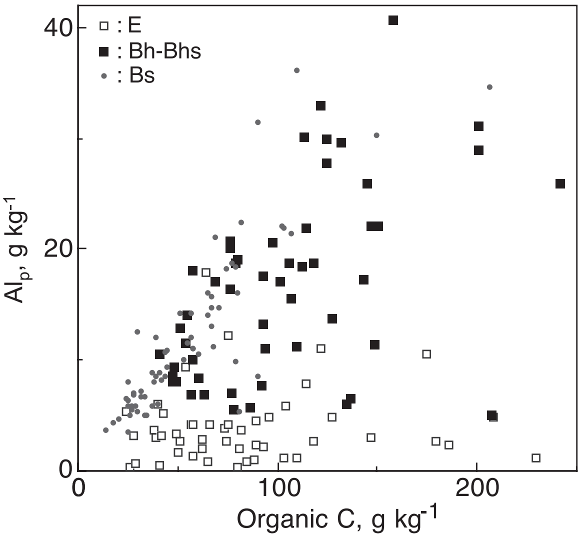

Fig. 4. Relationship between organic C content and pyrophosphate-extractable Al (Al_p) of Spodosols derived from tephra.

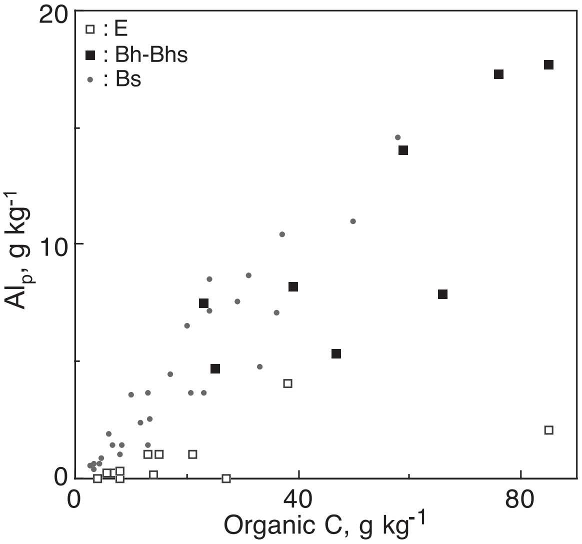

Fig. 5. Relationship between organic C content and pyrophosphate-extractable Al (Al_p) of Spodosols derived from nontephra.

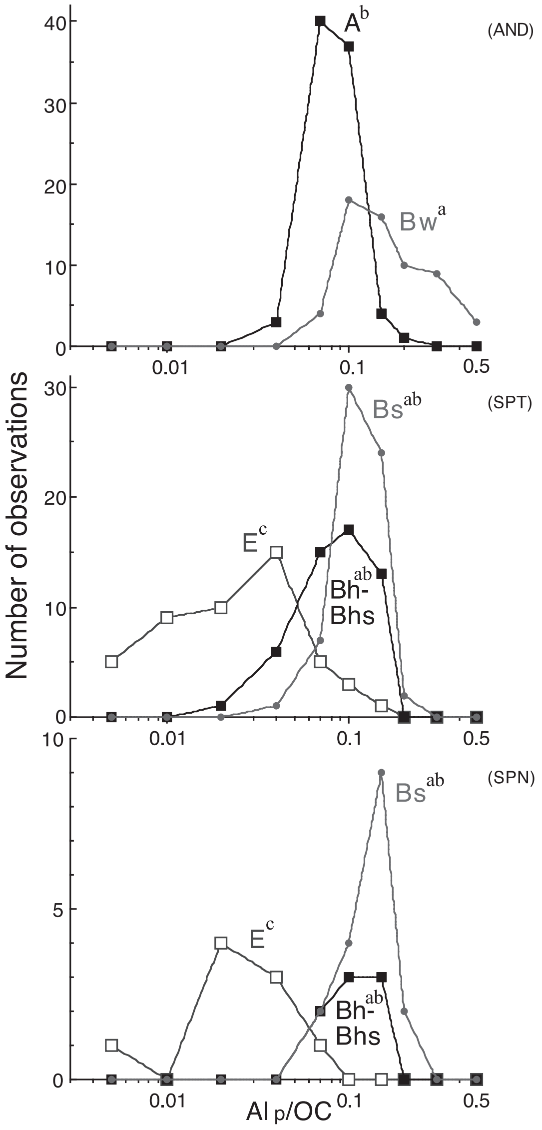

Fig. 6. Frequency distribution of atomic Al_p/OC ratio in the A and Bw horizon of Andisols (AND), E, Bh-Bhs and Bs horizon of Spodosols derived tephra (SPT) and those derived from nontephra (SPN). For example, a plot at 0.02 shows the number of observations for $0.01 < OC \leqq 0.02$. Mean values for the soil horizons followed by the same letter are not significantly different at p=0.05 level according to Scheffé's F test after logarithmic transformation.

of the Bh-Bhs horizon soils even after the logarithmic transformation. FD-Al_p/OC of the Bw horizon soils (AND) was not significantly different from those for the Bs of SPT and the Bh-Bhs and Bs of SPN. The A horizon soils of AND are not significantly different from those for the Bh-Bhs and Bs horizon soils of both SPT and SPN, although the Al_p/OC values for the Bh-Bhs and Bs horizon soils tends to be higher than those for the A horizon soil of AND. In contrast, the Al_p/OC values for the E horizons of both SPT and SPN are significantly lower than those for the other soil horizons. Thus, humus in the E horizon soils of both SPT and SPN has a lower degree of Al complexation than that in the other soil horizons. The degree of Al complexation in the E horizons appears similar (Fig. 6) between SPT and SPN although the OC content in SPT is higher than that in SPN (Fig. 2).

Almost similar relationships are obtained using $(Al+Fe)_p$/OC instead of Al_p/OC as indicated in Fig. 7.

The frequency distribution of the $(Al+Fe)_p/OC$ values of the E horizon soils is also lower than those for the Bh-Bhs horizon and Bs horizon soils for both SPT and SPN. Although the reasons for the reduced mobility of DOM may be microbial degradation of organic ligands, sorption of DOM in the Bh-Bhs and Bs horizons, etc., the resultant metal/OC ratio increases in the order E < Bh-Bhs < Bs horizon, and metal complexation may contribute to the stabilization of OM in the Bh-Bhs and Bs horizon soils.

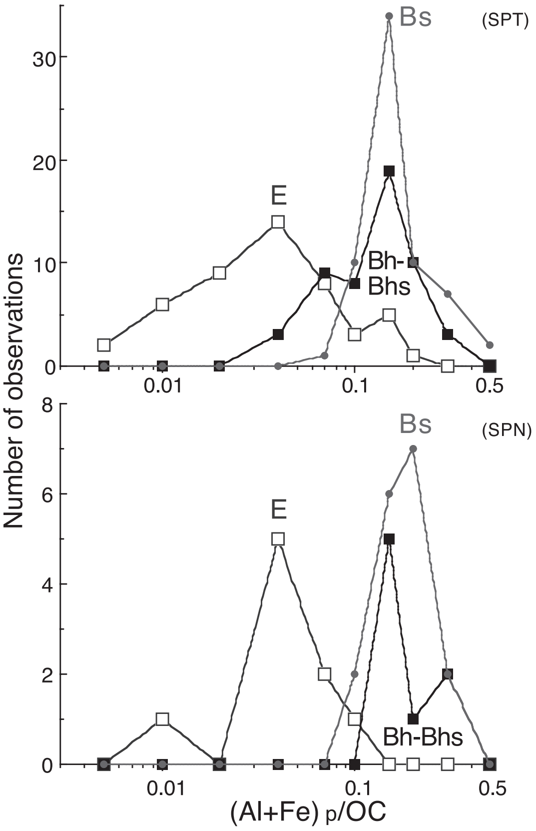

Fig. 7. Frequency distribution of atomic $(Al+Fe)_p/OC$ ratio in the A and Bw horizon of Andisols (AND), E, Bh-Bhs and Bs horizon of Spodosols derived tephra (SPT) and those derived from nontephra (SPN). For example, a plot at 0.02 shows the number of observations for $0.01 < OC \leqq 0.02$.

3. Content and chemical state of carboxyl groups evaluated from Al_p and CEC

Possible reasons for the low Al_p/OC and $(Al+Fe)_p/OC$ values of the E horizons are (i) washing effect by DOM to remove Al and Fe from this horizon soils, and/or (ii) low content of carboxyl groups to complex with Al or Fe. If the former is dominant, free carboxyl groups in the E horizons might be abundant. Smectite in the E horizon has little interlayering of the hydroxy Al polymer even under the low pH conditions (Shoji and Yamada, 1991). If the latter is more significant, the content of free carboxyl group would be low. As an index of the free carboxyl group content, the CEC of the different horizons was examined as a function of the OC.

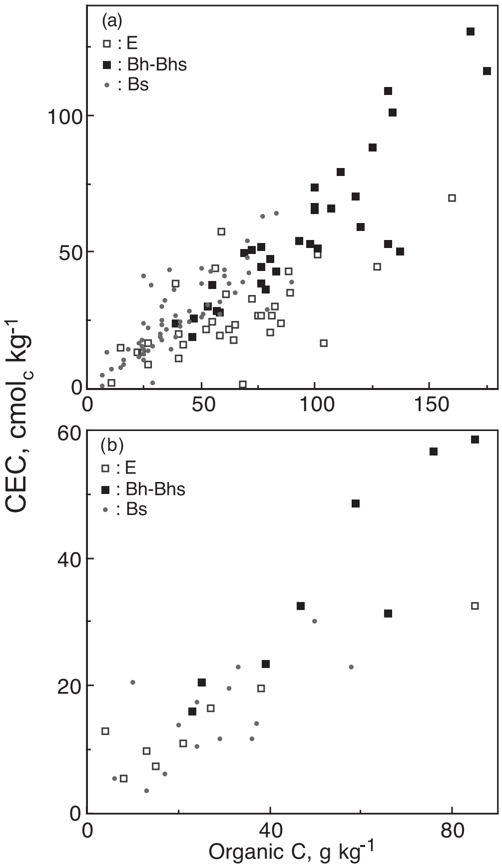

Fig. 8. Relationship between organic C content and cation exchange capacity (CEC) of Spodosols derived from tephra (a) and nontephra (b) by NH_4^+ acetate method.

The CEC values basically showed a linear relationship with the OC content for both SPT and SPN (Figs. 8a and b). The Bh-Bhs horizon soils were plotted nearly along the line for the Bs horizon soils. These relationships indicate that the OM of these soils has almost a similar degree of free carboxyl groups. In contrast, the CEC values for the E horizon soils of SPT were plotted in an area lower than those for the Bh-Bhs and Bs horizon soils. Thus, the total content of the carboxyl group, including both the complexed (Figs. 6 and 7) and free ones, in the E horizon soils of SPT is smaller than those for the Bh-Bhs and Bs horizon soils.

A similar result was obtained for SPN as shown in Fig. 8b. In the case of SPN, the E horizon soils were plotted nearly along the line for the Bh-Bhs and Bs horizon soils. A possible reason may be the higher contribution of smectic clays to the CEC of the SPN-E horizon soils than that of the SPT-E horizon soils as estimated from the data reported by Shoji and Yamada (1991). They reported that the SPN-E horizons have a mean clay content of 5.0 % (2.6-8.35, n=7), a mean OC content of 29 g kg^{-1} (4-85 g kg^{-1}, n=7) and a mean CEC value of 15.7 cmol$_c$ kg^{-1} (7.5 – 32.6, n=7) while the SPT-E horizon has clay 7.4 (3.9-11.5, n=7), OC 70 (42-82, n=7) and CEC 23.7 (16-30.3, n=7). Although the OC content of the SPN-E horizons is less than half the SPT-E horizons, the CEC of the SPN-E horizons is more than half the SPT-E horizons. The relatively high CEC of the SPN-E horizons is due to their clay content of more than half the SPT-E horizons. Thus, in the E horizon soils, the content of the total carboxyl groups, including the free and Al-complexed ones, is not higher than those for the Bh-Bhs and Bs horizon soils of the SPN.

Although not statistically significant, the Al$_p$/OC atomic ratio tended to be higher in the Bh-Bhs and Bs horizons of both the SPT and SPN than in the A horizon soils of AND (Fig. 6). The CEC values for the Bh-Bhs and Bs horizon soils of SPT and SPN (Fig. 8a and b) are also higher than those for the A horizon soils of AND (Fig. 9). The approximate ratios of CEC to OC of the Bh-Bhs and Bs horizon soils (Fig 8a and b) are higher than those for the A horizon soils of AND (Fig. 9). Hence, the total content of the carboxyl group in the Bh-Bhs and Bs horizon soils is greater than those for the A horizon soils of AND. A genetic reason for the high carboxyl group content is that the OM in the Bh-Bhs and Bs horizons were previously soluble or derived from the soluble OM with a high carboxyl group content. Both the chemical and biological processes, such as partial decomposition and/or sorption of DOM, are involved in the process of metal-humus complex formation in the Bh-Bhs and Bs horizons (Lundström et al., 2000). As the CEC (Fig. 9) and Al$_p$/OC values (Fig. 6) of the Bw horizon soils are higher than those of the A horizon soils, the OM in the Bw horizon may be partly translocated, although the OC content in the Bw horizon is far less than those in the A horizon soils (Fig. 2).

Regarding the CEC, the other difference in the A and Bw horizons of AND from the Bh-Bhs and Bs horizon soils of SPT and SPN is the y axis intercept of about 7.7 - 14 cmol$_c$ kg^{-1} (Fig. 9), while the y intercept is very low for the Bh-Bhs horizon soils (Fig. 8). The y intercept of A and Bw of AND is possibly due to the negative charge of clay minerals and other components.

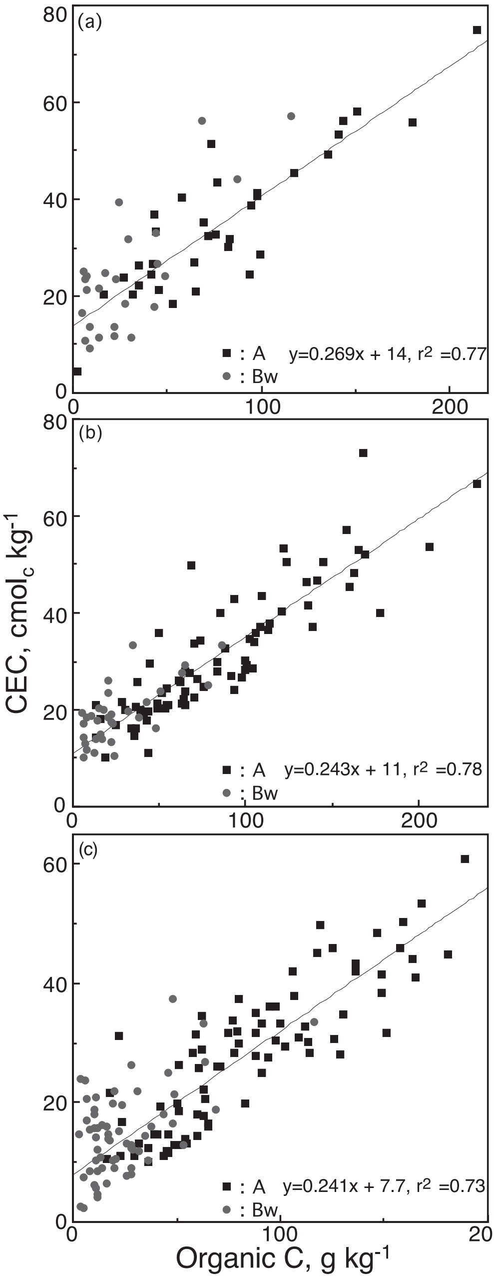

Fig. 9. Relationship between organic C content and cation exchange capacity (CEC) of Andisols determined by NH_4^+ acetate method in USA (a), by NH_4^+ acetate method in Japan (b) and by Ca^{2+} acetate method (c).

There are different methods to determine the CEC of soils. In the above discussion, the CEC was determined in the USA using NH_4^+ as an index cation at pH 7. These CEC values are close to those determined for AND by Japanese soil scientists basically using the same method (Fig. 9b). The CEC values determined using 0.05 mol L^{-1} Ca^{2+} (Wada and Harada, 1969) is also close to those determined by NH_4^+ acetate (Fig. 9). The same relationships shown in Fig. 8, Fig. 9a and b were also obtained using CEC by 0.05 mol L^{-1} Ca^{2+}. The CEC values by Ca^{2+} for the Bh-Bhs horizon soils of SPT is higher than those for the A horizon soils of AND (Figs. 9c and 10). The CEC values for the E horizon soils (Fig. 10) tend to be lower than those for the Bh-Bhs and Bs horizon soils of SPT (Fig. 10, Ping et al., 1989) and the A horizon soils of AND (Fig. 9c).

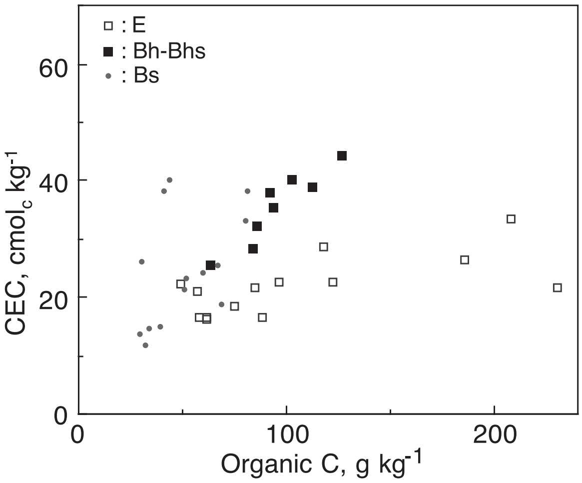

Fig. 10. Relationship between organic C content and cation exchange capacity (CEC) of tephra-derived Spodosols determined by Ca^{2+} acetate method.

The previous discussion about the fact that the Bh-Bhs and Bs horizon soils of SPT and SPN have more carboxyl groups than OM in the E horizon soils is compatible with the ^{13}C-NMR results (Kögel-Knabner et al., 2008). Kögel-Knabner et al. (2008) summarized that the content of the alkyl group decreases and carboxyl group increases in the order: EA < Bh < Bs horizon. The reason for the low Al_p, Fe_p and CEC values of the E horizon soils is the low carboxyl group content in the OM.

According to von Lützow et al. (2008), the ^{14}C ages of the E horizon soils are close to those for the Bs horizon soils and older than those for the Bh horizon soils. Metal-complexation in the E horizon soils is less than in the A, Bw horizon soils of AND and the Bh-Bhs and Bs horizon soils of SPT and SPN, and the content of the free carboxyl group is not very high. von Lützow et al. (2008) suggested hydrophobicity as a stabilization mechanism of OM in the E horizon although it was not adequately quantified by contact angle measurements. However, a slight Al complexation as well as a low pH may still be effective for stabilizing the OM in the E horizon resulting in its low solubility and low accessibility by microorganisms because the properties of the static OM in the E horizon is somewhat different from that in the Bh-Bhs horizon.

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International Workshop in Faculty of Agricultural Science and Field Science Center in Tohoku University 2008

Sustainable Yield and Population Conservation for Marine Organisms from the Point of View of Genetic Resources

Date: March 20(Thu)-21(Fri), 2008
Place: Lecture room No.10 in Graduate School of Agricultural Science, Tohoku University, Sendai, Japan

Program (Schedule)

20 March (Thu)

09:30-10:00	Opening ceremony (Outline of the Workshop) …Akihiro KIJIMA (Tohoku University, Japan)
10:00-11:00	Key note "Significance of intensive and extensive genetic analyses of natural populations in evolution and conservation" …Mutsumi NISHIDA (The University of Tokyo, Japan)
11:00-12:00	Key note "Conservation of adaptive genetic diversity: insights and challenges" …Juha MERILÄ (University of Helsinki, Finland)
12:00-13:30	Lunch
13:30-14:00	Population genetic study on the clam *Coelomactra antiquata* along the coast of China …Qi LI (Ocean University of China, China)
14:00-14:30	Kumamoto oyster (*Crassostrea sikamea*) is abundant in its native habitat, the northern Ariake Sea, Kyusyu …Masashi SEKINO (Fishery Research Agency, Japan)
14:30-15:00	Stock impact of exogenous hatchery-reared abalone in Japan …Motoyuki HARA (Fishery Research Agency, Japan)
15:00-15:30	Population genetic study of spiny king crab (*Paralithodes brevipes*): relationship between ocean current and patterns of stock structure …Minoru IKEDA (Tohoku University, Japan)
15:30-16:00	Coffee break
16:00-16:30	Population genetic studies on Echinoderms: population identification and stock management in Japan …Manami KANNO (Tohoku University, Japan)
16:30-17:00	Molecular-based ecological approaches for understanding migratory ecology of the freshwater eels…Jun AOYAMA (The University of Tokyo, Japan)
17:00-17:30	General discussion
18:00-20:00	Night Session

21 March : Excursion (9:00-17:00)
Inspection of Tohoku University and Matsushima Aquarium
22 March : Satellite Symposium-Open Seminar in Onagawa Field Center (13:00-16:00)

Opening remarks

Akihiro KIJIMA

Graduate School of Agricultural Science, Tohoku University, Japan

In Japan, a variety of fishery species including vertebrates, invertebrates and seaweeds etc., have been utilized as important foods from an ancient period. For example, Japanese eat not only finfishes, shellfishes and crustaceans but also echinoderms, acidians and others. Recently, it is well known that seafoods are quite good for health and elongation of a life, then many people are becoming eat them in the world. However, the fishery resources have decreased by overfishing and environmental impacts. It is necessary to take prompt measures for sustainable yield of fishery resources.

Naturally, fishery resources used to be unlimited renewable resources before excessive human activity (fisheries). But now, we have to manage the fisheries in the coastal and offshore area beyond the sovereignties. Currently, most of governments comply with TAC (total allowance catch), and are making efforts to improve the environmental condition such as spawning ground, nursing ground and fisheries area. Also, technology of artificial seed production and release them to the coastal area are developing for increasing fishery resources. These actions have been effective, and it could be basically essential, but still not sufficiently supportive. For reconciling conservation and production of fishery resources, genetic viewpoint should be necessary, because we have to think about continuous production beyond the generations.

To achieve sustainable fisheries in the world, it has been more essential to investigate the genetic state of the biological resources as well as their ecology. In this workshop, with the concept of the management of genetic resources, the roles and actual states of phylogenetic and population genetic studies on mollusca, crustacean, echinoderm and vertebrates are introduced and discussed.

Significance of intensive and extensive genetic analyses of natural populations in evolution and conservation

Mutsumi NISHIDA

Ocean Research Institute, The University of Tokyo, Japan

For achieving conservation and reasonable management of biological resources in marine and inland waters, it is very important to understand various aspects of their diversity, such as their evolutionary relationships, population structure and genetic diversity of species. Intensive and extensive genetic analyses of groups of species and natural populations of a species are essential for this. In the present workshop, I would like to discuss on this subject on the basis of our research experiences.

One of goals of our research team has been clarifying phylogenetic relationships of fishes, which are important components of aquatic ecosystems, aiming to understand their evolution at the gene and genome level. We would emphasize that reliable phylogenetic frameworks are indispensable bases for biologically meaningful comparisons, which are necessary for evolution and management studies. Reliability of traditional phylogenies is not so high, nonetheless, because they have been based on a limited amount of information. We have begun therefore to study molecular phylogenies on the basis of large amounts of nucleotide sequence data from complete mitochondrial genomes. We have mostly completed sequencing and analyzing of representative species from major families of ray-finned fishes, and have succeeded to provide a rather comprehensive phylogeny of them. Here, firstly I present an outline and some relevant examples of our results of this line of studies.

Another aim of our research team is to understand nature of species including genetic population structure of species. Through studies for this purpose, we have become aware that intensive and extensive genetic analyses of natural populations are extremely useful. Therefore, in this workshop, I introduce some examples of our studies showing that extensive sampling of specimens of species concerned from a wide range of distribution area is very useful to improve our understanding of fish species.

One of important direction of future study appears to be population genetic analyses of genes responsible for local adaptation of natural populations. We are trying to establish a methodology for identifying such genes by using a novel molecular biology technique. In the end of my talk, I will present our present stage of this trial and discuss future prospects.

Conservation of adaptive genetic diversity: insights & challenges

Juha MERILÄ

Department of Biological and Environmental Sciences, University of Helsinki, Finland

Genetic methods and information have assumed increasingly important role in conservation and management of wildlife. Most of the genetic data available for management and decision-making has traditionally consisted of information gained from neutral molecular markers, but more recently, data on ecologically important quantitative traits and genes coding these traits have gained increasing interest. In particular, comparative studies of neutral marker gene and quantitative trait divergence have provided interesting insights on conservation and management decision, but some practical and technical difficulties limit the utility of such comparisons. In my presentation, I aim to provide a short overview of what is currently known regarding the variation in genes coding quantitative traits among different populations of the same species. I will do this by focusing on comparative studies of quantitative genetic and marker gene differentiation, and reflect upon their utility in conservation and management context.

Population genetic study on the clam *Coelomactra antiquata* along the coast of China

Qi LI

Fisheries College, Ocean University of China, China

Coelomactra antiquata is a commercially exploited bivalve, and its populations have been severely declining in the coast of China during the last two decades. In order to provide guidelines for conservation strategies and management programs, four populations of *C. antiquata*, representing the most important populations in China, were analyzed morphologically and genetically to reveal the genetic structure by using 7 polymorphic allozyme loci and 12 morphological variables. All genetic distance metrics revealed high level of genetic differentiation between the three northern populations and one southern population. The marked genetic differentiation can be explained by the freshwater outflow of Yangtze River and upwelling of Zhejiang province. The morphological analyses were in accordance with the allozyme data, which also disclosed a high degree of differentiation between the northern and southern populations. The results obtained in this study indicate that the northern and southern populations in *C. antiquata* should be treated as separate units for conservation management.

Decline in the supply of wild caught *C. antiquata* has created an opportunity for the production of cultured clams and in the past three years, several commercial hatchery stocks are being developed in Shandong and Fujian Province, China. Understanding the genetic variation within hatchery stocks is needed for genetic enhancement programs aiming to avoid potential inbreeding, and random genetic drift. AFLP markers were used to investigate levels of genetic diversity within cultured populations of *C. antiquata* and to compare them with the wild source populations. High polymorphism at the AFLP markers was found within both cultured and wild *C. antiquata* populations. Although not statistically significant, reductions in the expected heterozygosity and percentage of polymorphic loci were observed in the cultured populations, and higher frequency of private alleles within the wild populations compared to the cultured populations indicated that rare alleles in some loci were lost in the cultured populations. Significant genetic differentiation was observed between the cultured populations, and between the cultured and wild populations. The results indicate that continued genetic monitoring of the cultured populations is warranted and the northern and southern populations of *C. antiquata* should be managed separately in hatchery practices for the preservation of genetic diversity in wild populations.

Kumamoto oyster (*Crassostrea sikamea*) is abundant in its native habitat, the northern Ariake Sea, Kyushu.

Masashi SEKINO

Tohoku National Fisheries Research Institute, Fisheries Research Agency, Japan

The Kumamoto oyster (Kumo, *Crassostrea sikamea*), which has taxonomically close relationship to the Pacific oyster (Pacific, *C. gigas*), is distributed exclusively in the Ariake Sea, Kyushu Island, Japan. Despite that Kumo has commanded little share of trade in the Japanese fishery/aquaculture, this species was first exported to US some sixty years ago and since then it has highly been valued by seafood connoisseurs and west coast oyster culturists as an aquaculture produce. From the view of conservation for the species diversity of oysters, it is of our great concern about the resource status of natural Kumo since previous expeditions in the native habitat have suggested that this species might be threatened, or if not, have reduced resource. In US, on the other hand, from the standpoint of aquaculture much attention has been paid to the maintenance of bona fide Kumos, as it has been shown that some introgression between Kumos and Pacifics occurs, prompting the need to introduce a new broodstock into the US aquaculture. Because of the morphological resemblance between Kumo and Pacific together with the phenotypic plasticity of oyster species in general, it is almost impossible to discriminate the two species on the basis of the morphology, thereby the resource status of Kumo has yet to be clear. Recent advent of molecular techniques, however, allows unambiguously discriminating Kumos from Pacifics. Using such molecular tools, we surveyed the northern parts of Ariake Sea to address the resource status of natural Kumo. We found that Kumo is far from rare species, or rather, it is the dominant oyster in the surveyed areas. In addition, a nuclear DNA marker provided no evidence of natural hybridization between the two species.

Stock impact of exogenous hatchery-produced abalone in Japan

Motoyuki HARA

National Research Institute of Aquaculture, Fisheries Research Agency, Japan

The Pacific abalone *Haliotis discus* species, including a cold-water distributed type called Ezo-abalone (*H. discus hannai*) and a warm-current inhabited type called Kuro-abalone (*H. discus discus*), is widely distributed in coastal areas of East Asia. Ezo- and Kuro-abalone are the most important abalone species for commercial fisheries resources owing to its high market value. Recent annual landing of these species have been reduced to about the 30% volume compared to the maximum catch level. This has led to an increase in hatchery abalone production from the 1980s, and a lot of hatchery abalone has been stocked intensively in coastal areas across Japan every year. Techniques for artificial production of Ezo-abalone juveniles are well established, but the production of Kuro-abalone juveniles has been pampered due in large part to the high mortality of seedlings caused by infectious disease. A growing concern underlies whether the mass-release of Ezo-abalone seedlings into the habitat of Kuro-abalone so as to increase the abalone resource is acceptable in the sense of the ecology, biology, and genetics of the resource. We studied the impact of releasing exogenous hatchery-reared juveniles on populations in Pacific abalone based on genetic markers at the population and individual level. Standard measures of genetic variation (R_S and H_E) for collections from Ezo- and Kuro-abalone habitats did not differ. However, samples from Ezo- and Kuro-abalone habitats, respectively, were clearly divided into genetically distinct clusters; a result that was consistent with the F_{ST} analysis. In population-assignment testing, most individuals collected from Kuro-abalone habitats, where intensive release of hatchery-reared Ezo-abalone juveniles have been carried out, were assigned to a genetically defined Kuro-abalone group, suggesting that most of exogenous hatchery-reared Ezo-abalone had difficulty reproducing successfully in Kuro-abalone habitats. However, in the warm-water sampling area stocked with the greatest number of exogenous Ezo-abalone hatchery-reared juveniles, the numbers of misassigned individuals were highest. This suggests that the reproductive contribution of stocked hatchery-reared Ezo-abalone cannot be ruled out completely. Therefore, hatchery-mediated introgression of exogenous Ezo-abalone genes into the Kuro-abalone subspecies remains a possibility. To support the goal of a genetically fit and sustainable abalone fishery, further detailed studies and evaluation of the potential impact of introgression are needed.

Population genetic study of spiny king crab (*Paralithodes brevipes*): relationship between ocean current and patterns of stock structure

Minoru IKEDA

Graduate School of Agricultural Science, Tohoku University, Japan

The spiny king crab *Paralithodes brevipes* (Japanese name: Hanasaki-gani) occurs off northern Japan in the Sea of Okhotsk and in the Bering Sea. It is an important fishery resource of eastern Hokkaido, but the catches have declined in recent years. Nemuro City in Hokkaido, well known for fishery of this crab, has organized the "Hanasaki Program" consisting of researchers from different study fields (ecology, oceanography, and population genetics, etc.) to survey this crab's biology for the resource management and stock enhancement toward sustainable use. We have joined in this program since 2005 in order to reveal population structure of this species around coastal areas of Hokkaido and Far East Russia. Using microsatellite and mitochondrial DNA markers, we revealed that remarkable genetic differentiation occurs between the southern side of the Sea of Okhotsk (the southern Sakhalin and northern Hokkaido) and the eastern side of the Pacific Ocean (the South Kuril and eastern Hokkaido). The presumable boundary between these two putative stocks is deemed to be the Nemuro Straight. The simulation on the dispersal of planktonic larvae based on the POM (Princeton Ocean Model) thoroughly explained the current larval dispersal within each stock and isolation between stocks. These results suggest that coupled molecular population genetic and ocean physical model analyses give us essential information to recognize management units (MUs) for the marine organisms having difficulty of tracing their actual larval transport in ocean.

Population genetic studies on echinoderms: population identification and stock management in Japan

Manami KANNO

Graduate School of Agricultural Science, Tohoku University, Japan

Echinodermata is one of the biggest phyla containing 5 classes (Sea cucumber, Sea urchin, Sea star, Brittle star and Sea lily), and found at all ocean depths and all latitudes. This group is a member of deuterostomes which is closely related to chordates, so it has been used for the embryology from the past. However on fisheries, it is very minor group in the world except Asian countries, so there is little genetic information for resource management at present. In our laboratory, we have been trying to make the basis of the population genetic study of echinoderms from the development of DNA markers. Here I introduce the three outcomes in our lab. about three echinoderm groups, a sea cucumber, a sea urchin, and two sea stars.

Japanese sea cucumber, *Apostichopus japonicus*, is the almost only one marketed species in Japan, and also popular fishery species in China, Korea and Russia. This species has three color variants, namely Red, Green and Black, however the taxonomic relationship among them has not long been clear because of few morphological traits of it. So we tried to evaluate the color trait quantitatively, and then investigated the genetic differentiation among them using allozyme and microsatellite DNA markers. As results, Red was clearly divided from the other color types, both in color trait and in genetic analysis showing the reproductive isolation of it. On the other hand, Green and Black showed continuous color distribution and no genetic difference suggesting the genetic homogeneity of them.

Strongylocentrotus nudus is a common sea urchin distributed around Japan. In order to detect the management units of it, we carried out mtDNA sequence analysis for the samples from 5 localities. As results, the typical haplotype networks suggesting the recent population explosion of the species were observed, and genetic compositions showed no statistic difference among the localities. Therefore, the present genetic structure which shows lack of genetic difference among the localities is thought to be strongly influenced by their recent historical expansion, and it is difficult to discuss about the present gene flow among the localities.

Asterina pectinifera and *Asterias amurensis* are common sea stars distributed along the coastal side of Japan. They have very similar life history but different taxonomic back ground. From the allozyme and mtDNA PCR-RFLP analysis, very contractive genetic structure was detected between them, which is genetic uniformity of *Asterina pectinifera*, and highly divergent genetic structure of *Asterias amurensis*. The difference in population structure of the two species was thought to be caused by the length of the history of the populations.

Through the introduction of the three studies, here I arrange the characteristics of the DNA markers on stock identification, and suggest the future subject for genetic management of echinoderms.

Molecular-based ecological approaches for understanding migratory ecology of the freshwater eels

Jun AOYAMA

Ocean Research Institute, The University of Tokyo, Japan

Recent progress in molecular techniques and establishment of mitochondrial DNA sequence database for all species of the genus *Anguilla* (Aoyama et al. 2001, Minegishi et al. 2005) have enabled us to genetically identify anguillid larvae (leptocephali), even eggs and newly hatched larvae that are difficult to identify under natural conditions since only limited number of morphological characteristics have been obtained in laboratory settings through artificially induced spawning. The leptocephali of many sympatric tropical species that are morphologically similar also have long been impossible to identify to species level, and their spawning areas are virtually unknown.

Recent findings relevant to the spawning areas of the genus *Anguilla* based on such molecular species identification are greatly changing our understanding of the migratory ecology and evolutionary history of these fascinating catadromous species.

The spawning area of the Japanese eel (*Anguilla japonica*) were recently determined as a pinpoint location near the Suruga Seamount (14°13.7N, 142°53.0E, Tsukamoto 2006), and small leptocephali of two tropical anguillid species, *A. celebesensis* (12.3 mm total length, TL) and *A. borneensis* (8.5 mm TL), were collected around Sulawesi Island, Indonesia (Aoyama et al. 2003). This finding indicates that much shorter migrations of a few hundred kilometers are made by tropical eels which were molecular phylogenetically suggested to be the most ancestral species, to spawn in areas near their freshwater habitats. This clearly contrasts with the long distance migrations of their counterparts in temperate regions, such as European *A. anguilla*, American *A. rostrata* and Japanese eels *A. japonica*, and suggests an evolutionary cline of migratory behavior in freshwater eels from tropical to temperate regions (Tsukamoto et al. 2002, Kuroki et al. 2006).

Introduction of New Project

Project of Integrated Compost Science

Graduate School of Agricultural Science, Tohoku University
Project Leader
Professor Yutaka Nakai, Ph.D

Vast amount of organic waste are being generated continuously by human activities in forests, hilly-mountainous areas, agricultural fields, coastal areas, and urban areas. The wastes accumulate in the field, and will result in not only global environmental pollution but also depletion of limited organic resources. I believe that a circulation system for the recycling and appropriate reuse of organic wastes should be developed breaking out of this situation as soon as possible. “Composting” is one of the suitable method for the circulation system, which has been traditionally implemented in Japan.

The compost processes in conventional composting methods can be a cause of ammonia volatilization resulting in acid rains. The application of large amount of composts with low nitrogen content result in excess assimilation of phosphoric acid in the field. In addition, there are concerns that these phenomena could cause eutrophication of water systems, thus leading to serious environmental destruction.

The acid composting technology for food wastes developed at Tohoku University inhibits ammonia volatilization and indicates the potential for developing environmentally friendly compost. However, only this acid composting technology cannot develop a recycling system for organic wastes. Compost safety should be assessed by analyzing the components of the compost produced. It is also necessary to assess both the functionality and availability by the actual applications of the compost. We have been investigating these issues at the Field Science Center, Graduate School of Agricultural Science, Tohoku University. Moreover, in terms of the cost performance, which is a major problem at the actual applications of the compost, we are trying to adjust the problem by analyses based on a standpoint of both social and natural sciences such as the satellite imaging, economics and LCA analysis.

Many laboratories in Graduate School of Agricultural Science have been studying compost for many years. In 2004, they initiated a project—“PICS” (Project of Integrated Compost Science)—in collaboration with several laboratories of the Graduate School of Engineering. In 2005, the Graduate School of Agricultural Science formulated an agreement with Miyagi Prefecture to establish a regional alliance research project—“PICS Miyagi.” The achievements of the project were accepted by the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan. MEXT awarded the PICS study entitled “Development of Ecological Recycling System for Organic Resources” a “Grant-in Aid for Special Education and Research on Integrated Collaborative Project.” This project will be conducted from fiscal year 2007–2011. In order to establish integrated compost science as a key factor in the recycling of organic resources, we conducted studies under this project by combining the research and development capabilities of our university. Further, we developed composting technology and investigated practical applications of this technology in cooperation with Miyagi Prefecture.

Furthermore, we studied the requirements of local communities. We promoted environmental education in these communities as well as technological transfer in these communities. We attempted to construct a model for a novel recycling system not only for local markets but also for global markets.

Background

In integrated ecosystems that range from forests to coastal areas including urban areas, a large variety and volume of organic wastes are generated by humans—the producers and consumers of organic substances. If organic waste is not properly disposed, it can easily cause environmental contamination such as soil contamination, nitrate contamination, or groundwater eutrophication. We cannot afford to dump the organic components in wastes because of the scarcity of natural resources in Japan. However, presently, there is no system for the effective recycling and appropriate reuse of organic wastes. Therefore, it is very important to work toward building a society that has a sustainable recycling system. Hence, it is essential to develop composting technology that is based on returning wastes to the soil and the reuse of organic components in wastes.

In Japan, composting has been traditionally implemented to recycle wastes produced by human activities. The process of conventional type composting method can result in acid rains due to ammonia volatilization. Because the conventional compost has low nitrogen content, a large amount of compost should be used, resulting in the excess phosphoric acid in arable fields. In addition, these phenomena cause unresolvable problems such as eutrophication of surrounding water systems. Moreover, the functions of compost components have not yet been thoroughly investigated, and the applications of composting technology have not been effectively determined. Therefore, the composting technology requires further development to be applied on a global scale.

It is important to establish systems for both the production and practical applications of compost. These systems should have minimum environmental burden and at the same time should be able to maximize the intrinsic functions of the compost.

Purpose

The purpose of this project is to effectively compost various organic wastes generated in integrated ecosystems; to establish an environment-friendly recycling system, which can be applied on a global scale; and to develop a research and education center for integrated compost science.

Our objective is to integrate disciplines such as pedology, environmental microbiology, and economics, which have traditionally been studied separately and to establish an academic system, which is based on the practical application of field sciences.

In combination with research facilities in Miyagi Prefecture and in other organizations, we train technical experts and researchers in highly advanced composting techniques and in integrated compost science.

Our aim is to raise the academic standards in Tohoku University as well as to promote its research achievements that are related to the recycling of organic wastes by utilizing large-scale research facilities in Miyagi Prefecture and other organizations.

1. Proposal of environmental friendly compost production methods, and the development of new composting methods to drastically reduce ammonia volatilization
2. Investigating the features of the compost produced (features that increase crop growth rate and that protect plants against pathogens)
3. Assessment of compost recycling

Composting Study Group

The Composting Study Group mainly conducts analyses on the acidulocomposting.

1) Analyses of the changes in microbial communities during acidulocomposting.

Dr. Tokuzo Nishino (previously a Professor at the Graduate School of Technology, Tohoku University; currently a Professor at the Tohoku Seikatsu Bunka College) and Dr. Toru Nakayama et al. (Graduate School of Technology, Tohoku University) developed an acidulocomposting system with thermoacidophilic microorganisms as seed bacteria. In this system, lactic acid bacteria stably exist as the dominant microbial species. The acidulocomposting system they developed was issued a patent entitled “The composting method of organic waste (patent number: 2001-220275).” We improved this acidulocomposting system and analyzed the functions of the microbial communities in this system. After analyzing the culture conditions, functional microbes are isolated. Gene analysis is an effective method to understand changes in microbial communities. Microbial communities were analyzed by denaturing gradient gel electrophoresis (DGGE). We also used the clone library method to identify the dominant microbial species.

2) Acidulocomposting by laboratory scale facility.

We examined the conditions for acidulocomposting that were suitable for the organic resources to be composted.

Composting Safety Research Group

1) Survey on the analytical methods and heavy-metal analysis for the safety involved in composting.

We ascertain the chemical properties of various composting materials by means of elemental analysis, quantitative analysis of the fertilizer components, etc. These data are utilized for the composting system design.

2) Examination of the effect of compost on aquatic insects.

There is concern that compost eluates may affect river ecosystems at the site of compost application. Therefore, in this study, we assessed the effect of compost eluate on aquatic insects.

- A literature review of the effect of chemical agents or other factors on aquatic organisms and insects in rivers.
- Selection of aquatic insects for the assessment of compost toxicity.

3) Analyses on the disinfecting effects of compost to pathogenic microbes.

We developed monitoring methods to understand the dynamic state of pathogenic microbes in the composting process and identified these pathogenic microbes. Moreover, although pathogenic microbes are killed by high temperature during the composting process, it is necessary to clarify other microbial factors such as bacteriocins, bacteriophages, and antibiotics.

Availability and Functionality Assessment Research Group

Composting is an appropriate method for the circular recycling of biological waste.

When compost is mainly used for agricultural applications, it is important to maintain a balance between the recycling of organic resources and sustainable production. To achieve the abovementioned objective, this group assessed the availability and functionality of the compost and developed an efficient and practical technology for the environment-friendly recycling of biological waste.

Research Themes and Achievements

Analyses of the characteristics and functionality of acidulocomposting

Compost derived from acidulocomposting (n = 12) was compared with that derived from raw garbage (n = 11). The following results were obtained.

(a) Low pH value (4.9 in acidulocomposting vs. 7.0 in garbage domposting)
(b) High nitrogen content (4.5 vs. 3.0)
(c) Low C/N ratio (12 vs. 17)

The above results indicated that the acidulocompost had a high organic nitrogen and that organic substances were degraded. Furthermore, inorganic nitrogen will be easily supplied. We applied both composts to soils and surveyed the germination and growth rate of Komatsuna (Brassica campestris). When the acidulocompost was compared with the aerobically produced compost from garbage, most of the raw compost derived from garbage lowered the plant growth rate. This observation indicated the presence of organic substances that inhibited plant growth.

Compost Comprehensive Assessment and Study Group

- Study on comprehensive methods for the assessment of compost and on practical applications of organic resources

In our project “Development of Ecological Recycling System for Organic Resources,” a resource recycling system is constructed by assessing the safety, functionality, and availability of compost. We characterize “the recycling system on organic resources for global symbiosis” and comprehensively assess the system.

1) Development of a technology for the economic efficiency assessment, including environment-friendly effects evaluated by LCA

Life cycle assessment (LCA) is a method used to quantify the environment burden of products generated during their life cycle, i.e., from production to disposal, which is mentioned as “from cradle to grave”. Products or services that have an economic cost of production but a high environmental burden are not beneficial to the environment. We develop assessment technologies for the availability and the economic efficiency including the assessment by LCA. The economic efficiency assessment is conducted in cooperation with Composting Study Group.

2) Development of a broad-based assessment technology used to determine the amount of organic resources generated using remote sensing

The total mass of organic material that is produced by living organisms, i.e., biomass, has so far not been accurately quantified. Therefore, we developed a broad-based assessment technology to evaluate the amount of biomass produced by various scales of urban development such as prefectures, municipalities, and settlements by remote sensing.

■コンポスト化研究部会 Composting Study Group

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■コンポスト化安全性研究部会 Composting Safety Research Group

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