Nitrogen and oxygen isotope fractionation during anammox in the activated sludge

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Anammox is an important nitrogen removal pathway in many ecosystems. However, it is still unclear how important the anammox is quantitatively compared with the denitrification. Natural abundance of 15N and 18O of nitrogenous compounds such as ammonium, nitrate and nitrite can provide unique infromation to investigate the relative contribution of anammox to the total nitrogen removal, although the lack of reports on isotopic fractionation factors in the anammox cannot allow us to explore the use of stable isotope signature in the anammox studies.
We incubated the sludge anaerobically to trace the changes in concentrations and isotopic signatures of ammonium, nitrite and nitrate during the anammox process to calculate the isotopic fractionation factors. We found the large isotopic fractionations for ammonium oxidation and nitrite reduction by anammox. In addition, the inverse isotopic fractionation during nitrite oxidation to nitrate was observed. Moreover, the exchange rate of O atom between water and nitrite was higher than previously thought, indicating the importance of this process in regulating the isotope systematics. In the presentation, wapply the isotopic fractionation factors obtained from our incubation experiments to make a simple process model to see if isotopic signatures can detect the anammox in the denitrification-dominated environment.

Keywords: Anammox, d15N, d18O

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Screening of metal-ion inducible genes from subseafloor sedeiments of Nankai Trouph using substrate-induced gene expression method

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There are still large room for discovery in gene function and its potential of life in natural environment especially for the least-explored environments such as the subseafloor biosphere. We have employed substrate-induced gene expression (SIGEX) approach to screen DNA fragment that induce gene expression as a response to presence of substrates such as metal ions, D-amino acids, and persistent chemicals from metagenomic shotgun libraries prepared with subseafloor sediment samples of Nankai Trough.
Constructed metagenomics shotgun libraries that confers *gfp* gene downstream of the inserted DNA fragment were induced by metal ions including Ni^{2+}, Co^{2+}, Mg^{2+}, Mn^{2+}, Mo^{6+}, and Ga^{3+}, followed by sorting of clones with green fluorescence by a cell sorter. From sorted clones, we secondary screened clones for its response to the induction of each metal ion. Clones isolated by using Ni^{2+}, Ga^{3+} showed specific response to each metal ion used for isolation. Also there were other type of clones that showed response to induction by multiple metal ions. DNA sequence analysis revealed that while around half of the clones had database-identifiable DNA fragment, other half did not show any match to DNA sequences in database. This study showed great potential of SIGEX-based approach to find function of DNA fragments from large pool on unknown environmental DNA of natural environment.

Keywords: Deap-sea sediment, Gene function, Nankai Trough, Metagenome, SIGEX, Metal-ion

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Predominant but previously-unseen prokaryotic drivers of reductive nitrogen transformation in paddy soils, unveiled by metatranscriptomics.

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Waterlogged paddy soils possess anoxic zones in which microbes actively induce reductive nitrogen transformation (RNT). In the present study, a shotgun RNA sequencing analysis (metatranscriptomics) of paddy soil samples revealed that most RNT gene transcripts in paddy soils were derived from *Deltaproteobacteria*; in particular, the genera *Geobacter* and *Anaeromyxobacter*. Despite the frequent detection of their rRNA in paddy soils, their RNT-associated genes have been rarely detected by previous PCR-based studies. Therefore, the present metatranscriptomics has provided novel insights into the diversity of RNT microbes present in paddy soils as well as the ecological function of *Deltaproteobacteria* predominating in such soils.

Keywords: Reductive nitrogen transformation, Deltaproteobacteria, Metatranscriptome, Soil ecosystem, iron-reducing bacteria

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Effects of urea application on the forest floors in limestone and serpentinite soils in the Kanto region, Japan

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Urea applications have been done in different habitats in acidic soils, which are common to Temperate and Subtropical Zones, in order to examine nitrogen cycling mechanism after disturbance by nitrogen enrichment. In Japan, most terrestrial plant communities are established on acidic soils since acidic soils distribute widely whole areas of Japanese archipelago. For example, pH and moisture content as well as NH_4-N concentration rise rapidly after urea application on forest floors in acidic soils. While, the soil pH returns to the control level (non-urea application level) within 7 months whereas NH_4-N concentration and water content decline slowly to the control level for 1-2 years after the urea application. Successional occurrence of a group of fungi was observed following the urea application and they were defined as ammonia fungi (a chemoecological group of fungi; Sagara 1975). The early phase ammonia fungi occur during high pH, high NH_4-N concentration, and high water content whereas the late phase ammonia fungi occur during the latter two factors still somewhat higher than the control. Based on the observation described above, a sudden increasing of NH_4-N associating with that of pH have been speculated as the essential factors for the occurrence of ammonia fungi (Yamanaka 1995; Suzuki *et al.* 2002, 2003; He & Suzuki 2004, etc.). However, nitrogen enrichment has not yet been done in the terrestrial plant communities established on alkaline soils such as limestone and serpentinite soils which distribute in Japanese archipelago. Therefore, we applied 800 g/m^2 of urea on the forest floor near Nippara (Consisting of a layer of re-transferred limestone soil deposited on the siliceous base and its clayey deposited layer; Okutama region, Japan) and that along the Mineoka trail (Consisting of serpentinite base and its clayey deposited layer; Kamogawa region, Japan) in the end of March in 2016. We examined the changes in mycobiota and physicochemical factors (soil temperature, water content, pH, and inorganic nitrogen concentrations) in the forests after the urea application. pHs of the non-urea- treated soils (LF and HA horizons) of the former and the latter were ca. 8 and ca. 10, respectively. NH_4-N concentration and moisture content of the soils rapidly increased in the urea plots of both sites and then gradually declined. The declining of NH_4-N concentration in the alkaline soils was quicker than that of NH_4-N concentration in acidic soils. In contrast, soil pH of the alkaline soils showed no significant changes as far as one year after urea application. After urea application, NO_2-N concentrations of HA horizons in the alkaline soils increased remarkably comparing with those of HA horizons in acidic soils subsequent to urea treatment. The difference in nitrification ability could be one of characteristics of the alkaline soils. The ammonia fungi recorded in urea-treated acidic soils, such the early phase ammonia fungi *Amblyosporium botrytis*, *Pseudombrophila petrakii*, *Coprinopsis echinospora* and *Lyophyllum tylicolor,* and late phase ammonia fungi *Calocybe constricta* and *Hebeloma spoliatum* were observed in the alkaline soils by the urea application, but not collected any fungal species specific to the alkaline soils. From the above results, it was suggested that disturbance due to a large amount of NH_4-N is mainly responsible for the propagation of ammonia fungi irrespective of presence and absence of rapid rising of pH.

Keywords: ammonia fungi, alkaline soils, Limestone, Serpentinite, pH, anmmonium nitrogen

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Microbially induced Smectite-to-Illite reaction

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Microbial Fe-reduction in smectite structure plays a significant role in illitization accompanying with the structural/chemical modification of smectite, closely linked to the physico-chemical properties of clays, Fe-liberation, water chemistry, elemental cycles, and fault behavior. Especially current researches exploring microbial diversity in the Nankai Trough fault and the influence of illitization on fault behavior emphasize the significance of the microbially induced smectite-to-illite reaction. The dissolution of smectite induced by microbial respiration of Fe in the structure of smectite is a major process that promotes illitization, however direct evidence of K-fixation and K-nontrontie/illite formation is not clearly understood.
The present study demonstrates evidence of biotic illitization during the reductive dissolution of nontronite (NAu-1) associated with microbial Fe-respiration, by the microscopic/spectroscopic measurements of progressive modification in morphology, structure, and elemental composition of bio-reduced nontronite as well as aqueous chemistry in the supernatant as incubation time increased. Fe-reducing bacteria (FeRB), *Shewanella Oneidensis* MR-1 was inoculated in M1 medium with nontronite (NAu-1) less than 0.2 micron as an electron acceptor and Na-lactate as a sole electron donor at 30 degree-Celcius in the anaerobic chamber. The pH was buffered with potassium-phosphate buffer at pH 7.0 and 8.0 for optimum condition for microbial growth and illite formation.
The progress of bio-reduced nontronite reaction can be explained as follows: altered nontronite (AN) with a scouring surface texture →K-nontronite (KN) with frayed edges →euhedral lath shaped illite. A progressive morphology change in bio-reduced nontronite corresponded to an increase in Al/Si and K/(K+2Ca) that ranged between 0.13 to 0.28 and 0.16 to 1.0, suggesting the biotic reductive dissolution of nontronite and neoformation of illite.
The changes in oxidation state of structural Fe and its consequences on the petrophysical properties of clay minerals during the illitization may modify the fault behavior and geological environments.

Keywords: smectite-to-illite reaction, Microbial Fe reduction, Fault property

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Microbial community in brucite-carbonate chimneys discovered in the Shinkai Seep Field, the deepest serpentinite-hosted vent system in the Southern Mariana Forearc

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Serpentinite-hosted fluid vent systems have attracted great interest as unique modern deep-sea chemosynthetic ecosystems and as analogues for the origin and early evolution of early on Earth as well as for extraterrestrial life such as on Mars and Enceladus. During expeditions since 2013 to 2016, brucite-carbonate chimneys were discovered from the deepest known (~5700 m depth) serpentinite-hosted ecosystem –the Shinkai Seep Field (SSF) in the southern Mariana forearc [1]. Here we report geobiological characteristics of the SSF chimneys, as a new type of chemosynthetic microbial habitat at a serpentinite-hosted vent system.

Previous explorations of SSF led to the discovery of fourteen vesicomyid clam colony sites and five chimney sites occurring within an area of 500 square meters. Textural observations and geochemical analysis reveal three types (I-III) of chimneys formed by the precipitation and dissolution of constituent minerals [2]. Type I chimneys are bright white to light yellow, have a spiky crystalline and wrinkled surface with microbial mat and mainly consist of brucite; these formed as a result of rapid precipitation under high discharge conditions of alkaline fluid. In this type of chimneys, filamentous microbial cells were often mineralized by brucite. Type II chimneys exhibit white to dull brown coloration, tuberous fluid pass textures, and are covered with grayish microbial mats and colonies of *Phyllochaetopterus*. This type of chimney is characterized by inner brucite-rich and outer carbonate rich zones and is thought to have precipitated from lower fluid discharge conditions than type I chimneys. Type III chimneys are ivory colored, have surface depressions and lack living microbial mats or animals. This type of chimneys mainly consist of carbonate, and are in a dissolution stage after alkaline fluid input ceased.

Small subunit rRNA gene tag sequences showed that prokaryotic community compositions varied with the chimney types, reflecting the hydrologic and biogeochemical processes. For example, alkaliphylic bacteria were abundant in type I chimneys and diverse symbiotic bacterial strains were identified in type II chimneys. The former likely reflects higher flux of alkaline fluid, whereas the latter possibly reflects higher biomass of faunal community on type II chimneys. Unique characteristics observed in the SSF chimneys shed light on the variability of subseafloor and seafloor geochemical and geobiological processes supporting the serpentinite-hosted exosystems.

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Keywords: Shinkai Seep Field, Serpentine-hosted vent system, Mariana Forearc, Chemosynthetic ecosystem, Deep-sea chimney

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Interaction between photochemical and microbial degradation of dissolved organic matter in the Pearl River Estuary

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The migration and fate of dissolve organic matter (DOM) in estuarine ecosystems have gained more attention, due to the important role of the estuary as the bridge of the land and ocean,. Photochemical and microbial processes have long been considered as the key routes for the transformation and mineralization of DOM in the aquatic ecosystems. However, how the above processes influencing each other is still poorly understood. In this study, the interaction between photo- and bio-degradation of DOM was investigated in surface water in the Pearl River Estuary in south China. Microbial, photochemical and microbial + photochemical experiments were carried out and monitored by direct measurements of dissolve organic carbon (DOC) and the adsorption spectra of chromophoric dissolved organic matter (CDOM). The results demonstrated both the fraction of microbial degradation of DOM and the rate of this pathway in the dark were higher in the mid estuary than those in the upper and lower estuaries . The microbial degradation of CDOM under natural light along the estuary was promoted, whereas that of DOC was inhibited. After one-month microbial consumption in the dark, the photobleaching rate of CDOM was slightly enhanced along the estuary. Differently from CDOM photobleaching, the photodegradation rate of DOC was promoted in the upper estuary, but inhibited in the lower estuary. Furthermore, two broadband shoulders at 285-310 nm and 350-360 nm, respectively, were found for the absorption spectrum of CDOM after microbial and then photochemical degradation in the upper estuary, which meant the formation of carbonyl and aromatic heterocyclic compounds. The shoulders were smaller (absent) for the mid (lower) estuarine sample. This study provides direct evidence that microbial and photochemical degradation of DOM can significantly impact on each other in the estuarine ecosystem. Further studies are needed to explore the mechanisms between the two processes in large scale areas.

Keywords: Pearl River Estuary, Dissolved organic carbon, Chromophoric dissolved organic matter, Photochemical degradation, Microbial degradation

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Water circulation between solum and weathered layer examined by Tank Model analysis related to weathering reaction

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Tank Model is a discharge analysis model to make an accurate estimate of low water discharge from the river basin. Solum covering the ground, had been built up as the result of activity of natural vegetation and microbe. The physical property of solum is characterized by large porosity and small density contrary to the weathered layer. Difference of physical property between solum and weathered layer corresponds to first tank and second tank in Tank Model. In the study we prepared model distribution of one day precipitation from average return period of one day precipitation. And with the model distribution of one day precipitation, we calculated infiltration, upper migration of water caused by transpiration and storage in each tank, applying Tank Models which represent the discharge of River Ayusawa and River Kawauchi in the same Sakawa river system. Mode of water circulation cycle between first tank and second tank caused by regular intervals of small rain and fine day was presented using the model distribution of one day precipitation. The result of Tank Model calculation in case of River Kawauchi, 1000mm average annual precipitation, is showed in Fig.1 Carbon dioxide was produced as the result of activity of microbe in the A layer, and dissolved into water in first tank . Then it was transported into second tank by unsaturated infiltration. At first cycle of infiltration, density of hydrocarbonic acid ion is diluted by remaining water in the second tank caused by prior big rain. But dilution rate of density of hydrocarbonic acid ion decrease at next cycle and the initial density is kept at third cycle. Increase of the cycle number will bring acceleration of chemical weathering.

Keywords: solum, water circulation, Tank Model, Sakawa River System, average annual precipitation, chemical weathering

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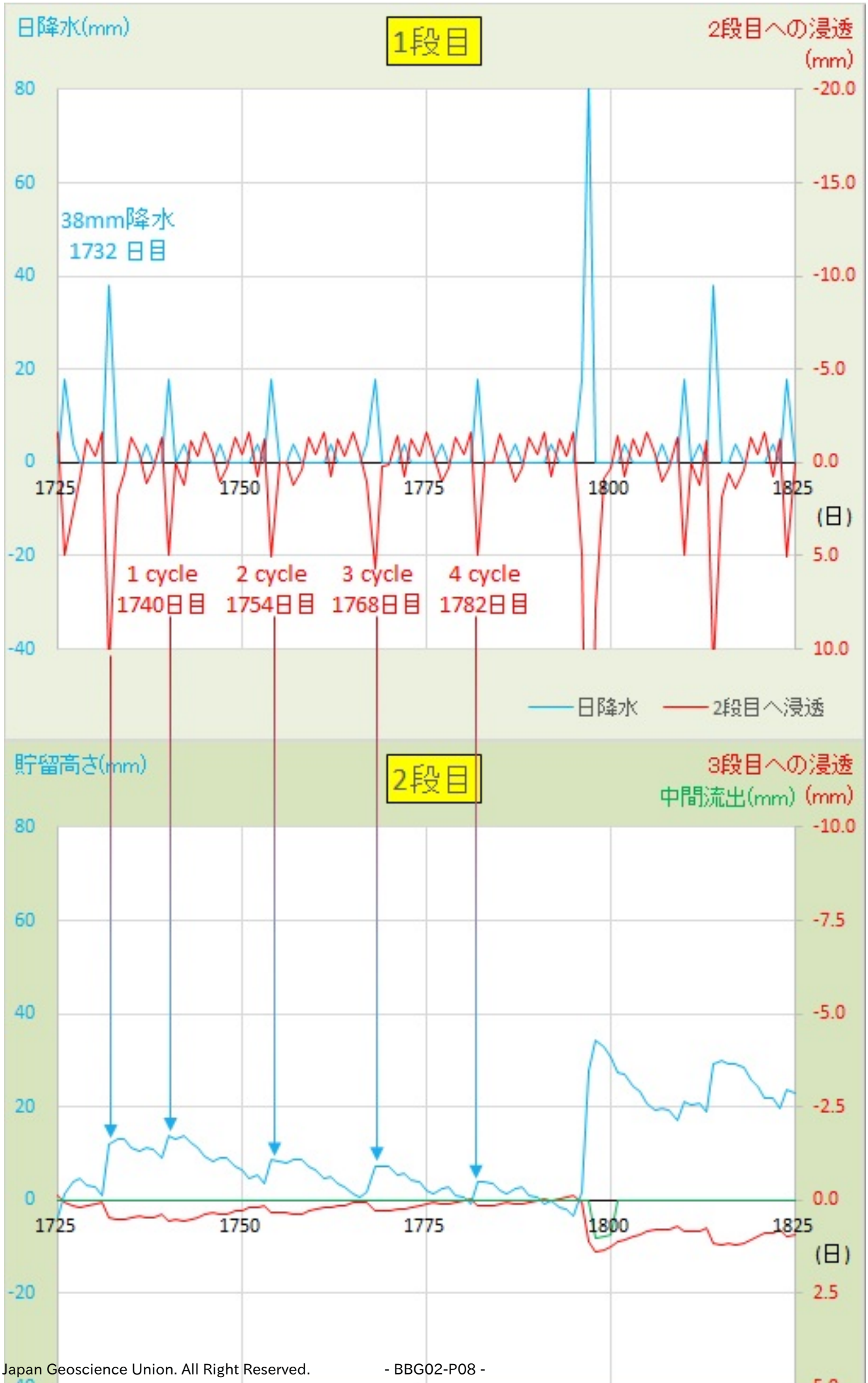

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